GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

Volume 24 MARCH, 1939 Number 2

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PUBLISHED BIMONTHLY AT MENASHA, WISCONSIN
BY THE BROOKLYN BOTANIC GARDEN
BROOKLYN, NEW YORK, U.S.A.

(Date of issue, March 25, 1939)

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Volume 24 MARCH, 1939 Number 2

GENETICS is a bimonthly journal issued in annual volumes of about 600 pages each.

Subscription, $6.00 net a year for complete volumes (January–November). Parts of volumes are to be had only at the single number rate. Foreign postage, 60 cents additional. Single copies, $1.25 each, postpaid.

Back volumes, as available, may be had at $7.00 each, plus postage. The Business Manager will supply information on request as to odd volumes and numbers and complete sets available.

Correspondence concerning editorial matters should be addressed to the EDITOR OF GENETICS, 804 Schermerhorn Hall, Columbia University, New York.

Business Correspondence, including change of address, directions concerning reprints, and exchanges, should be addressed to GENETICS, Menasha, Wisconsin, U.S.A., or BROOKLYN BOTANIC GARDEN, 1000 Washington Ave., Brooklyn, N.Y., U.S.A.

Remittances should be made payable to GENETICS.

Entered as second-class matter, August 31, 1922 at the post-office at Menasha, Wisconsin, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in the Act of February 28, 1925, paragraph 4, section 412, P.L. & R., authorized January 9, 1932.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

FURTHER TESTS OF THE RÔLE OF COSMIC RADIATION IN THE PRODUCTION OF MUTATIONS IN DROSOPHILA MELANOGASTER

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Received August 22, 1938

INTRODUCTION

THE rôle of cosmic radiation in the production of mutations in *Drosophila melanogaster* had been tested previously (JOLLOS 1937) by comparing the mutation rate of pure inbred stocks which were kept at Madison, Wisconsin and for various lengths of time on top of Pikes Peak, Colorado. It remained to be determined whether the difference in the intensities of cosmic radiation at these two different altitudes would produce a difference in the frequencies of mutations in sister cultures from the same stock. It was hoped that the results of such tests would perhaps show if and to what extent the so-called "spontaneous" mutations could be related to the influence of cosmic radiation.

The results of the tests made in 1936 differed for different periods of exposure on Pikes Peak. No significant increase in the mutation rate was found following an exposure for 28 days in September 1936. But cultures of the same stocks which were exposed for 24, 40 or 44 days in July–August showed a significant increase in their mutation rate. The cultures were kept, unintentionally, during July-August, but not during September, in a metallic environment (under an observation tower and directly behind a cash-register) which seems likely to favor the production of secondary radiation. Thus it was concluded that the ordinary five–six fold increase in the intensities of cosmic radiation at an altitude of about 14,000 feet over those at Madison altitude is, under the conditions of the test, not sufficient to increase the mutation rate significantly. But a further increase in the frequencies of secondary radiation by the passage of primary radiation through heavy metals seemed to produce significant positive results.

It seemed necessary to test this hypothetical explanation of the various results from different exposures in 1936 by further experiments, and to find out whether the comparison of more data concerning the mutation rate of pure inbred stocks of *Drosophila melanogaster* at different altitudes

[1] Investigations supported by a grant-in-aid from the Committee on Radiation, National Research Council. Laboratory facilities were offered by the University of Wisconsin and the University of Denver. The author is indebted also to Prof. F. D'Amour and Prof. J. Stearns of the University of Denver for their help during the course of the experiments on Mt. Evans; to Prof. Stearns for information and advice concerning the physical side of the problems; to Dr. A. B. Chapman of the Department of Genetics, University of Wisconsin, for his aid in the statistical calculations; and to Mrs. V. Jollos and Mr. E. Waletzky for technical assistance.

Second Printing 1966 / University of Texas Printing Division, Austin

within and without a metallic environment could show a quantitative relation between the intensities of cosmic radiation and the frequencies of mutations.

EXPERIMENTAL AND STATISTICAL METHODS

The methods used in these new tests were in general the same as in the previous experiments (JOLLOS 1937). Numerous cultures of two pure inbred wild stocks ("Oregon"[2] and "Lausanne") were kept for various lengths of time at an altitude of about 14,000 feet. Sister cultures were left at Madison. It was intended to run most of the high altitude series on top of Mount Evans instead of Pikes Peak, as the new Mount Evans Laboratory of the University of Denver and the Massachusetts Institute of Technology offered better facilities for the experiments. But road conditions made the Mount Evans laboratory practically inaccessible for the latter part of the summer of 1937. Thus only one series of exposures was run on Mount Evans, the others again on top of Pikes Peak. The altitudes of both places are nearly the same. Part of the cultures were kept far away from metallic surroundings; others were covered with lead plates of 18 mm thickness in order to create most favorable conditions for the formation of secondary radiation. Furthermore, a number of cultures were kept at Pikes Peak in the metallic environment where exposures in 1936 had given positive results.

All cultures were protected against ultraviolet rays, and kept within ranges of temperature which do not influence the mutation rate of Drosophila stocks significantly (JOLLOS 1937).

Stocks were exposed to the high altitude conditions for the following periods:

Period 1. Mt. Evans July 2–July 27. 25 days.
Exposed cultures: Oregon, Oregon-lead, Lausanne, Lausanne-lead.
Pikes Peak July 6–July 31. 25 days.
Exposed cultures: Oregon-M (in metallic environment).

Period 2. Pikes Peak August 1–September 9. 40 days.
Exposed cultures: Oregon, Oregon-lead, Oregon-M. Lausanne, Lausanne-lead.

Period 3. Pikes Peak September 9–October 9. 30 days.
Exposed cultures: Oregon-lead, Lausanne-lead.

The influence of the lead cover at Wisconsin altitude was tested for

Period 4. Madison December 18, 1937–January 17, 1938. 30 days.
Exposed cultures: Lausanne-lead, Oregon-lead.

It seemed necessary to start the experiments on a very large scale in

[2] The Oregon stock was the same as used in 1936.

view of the high mortality or sterility which was observed in cultures exposed to the high altitude conditions in 1936. Ten vials, each containing 15 freshly hatched males, and 40 vials (or bottles) with six fertilized females in each, were exposed for each period and each series. (Oregon, Oregon-lead, Oregon-M, Lausanne, Lausanne-lead.) Thus the tested X chromosomes of exposed males were under the influence of higher frequencies of cosmic radiation either only in the adult stage of the male, or in the adult stage as well as during the whole development of the male and also in the body of his mother. These differences in the stages which were exposed to the influence of cosmic radiation did not lead to differences in the mutation rate, as had already been found in similar cases in the previous experiments.

Only sex-linked lethal or semilethal mutations were determined by mating exposed males (or control males) with virgin ClB females, and examining the sex ratio in the offspring of Bar-eyed F_1 females mated with males from untreated stocks. Only those cases were considered as demonstrations of the presence of a semilethal sex-linked mutation, where a ratio of at least five females to one male was found in a minimum of 30 flies, instead of an expected ratio of two females to one male. Statistical calculations show that the choice of a five to one sex-ratio (in a minimum of 30 and a minimum average per series of about 50 flies) as indicator for a semilethal sex-linked mutation, gives sufficiently complete and reliable information concerning the frequencies of such semilethals in the tested flies.

The probability of a chance deviation from a two to one ratio to a ratio of five to one is, under these conditions, below 0.01 for the average and only 0.035 even for the very rare borderline cases, which showed a sex-ratio of 25 females to five males. A ratio of four females to one male which was used as indicator for semilethal sex-linked mutations in the tests in 1936 (and primarily also for the tests presented in this paper), seems sufficiently reliable for the average cases, but somewhat doubtful for borderline cases with a sex-ratio of 24 females to six males; the probability of such chance deviations from a two to one ratio being 0.028 and 0.084 respectively. The four to one sex-ratio, therefore, was abandoned as indicator for sex-linked semilethals in favor of a five to one ratio. As a matter of fact, the results were not altered significantly by the use of this more reliable indicator. Furthermore, the results are consistent if we consider the frequencies of lethal mutations under the various conditions of the experiments and those of lethals and semilethals (see below).

THE FREQUENCIES OF LETHAL AND SEMILETHAL SEX-LINKED MUTATIONS

The frequencies of sex-linked lethal and semilethal mutations in the offsprings of exposed and control males are shown in table 1.

TABLE 1

Frequencies of lethal and semilethal sex-linked mutations.

STOCK AND PERIOD OF EXPOSURE	NUMBER OF TESTED CHROMOSOMES	LETHALS		SEMI-LETHALS	% LETHALS AND SEMI-LETHALS	DIFFERENCE TESTS AND CONTROLS†		PROBABILITY FOR	
		NUMBER	PERCENT			LETHALS	LETHALS AND SEMI-LETHALS	LETHALS	LETHALS AND SEMI-LETHALS
Controls (at Madison)									
Oregon	995	4	0.40±0.200	6	1.01±0.317	—	—	—	—
Lausanne	1058	4	0.39±0.194	6	0.96±0.303	—	—	—	—
Period 1 (July)									
Mt. Evans 25 days									
Oregon	1004	6	0.60±0.244	9	1.49±0.382	0.63	0.97	0.53	0.33
Oregon lead	1356	14	1.03±0.274	27	3.24±0.481	1.86	3.87	0.065	0.0001
Lausanne	1049	7	0.67±0.252	9	1.62±0.390	0.88	1.34	0.375	0.18
Lausanne lead	1052	14	1.33±0.353	21	3.33±0.553	2.33	3.76	0.02	0.001
Pikes Peak 25 days									
Oregon M	1009	10	0.99±0.312	16	2.58±0.499	1.59	2.66	0.11	0.01
Period 2 (Aug.-Sept.)									
Pikes Peak 40 days									
Oregon	1249	8	0.64±0.226	13	1.68±0.364	0.79	1.39	0.43	0.165
Oregon lead	1384	22	1.59±0.336	32	3.90±0.520	3.04	4.75	0.0015	0.000001
		(19)	(1.37±0.312)*	(32	3.68±0.506)	(2.95)	(4.69)	(0.001)	(0.000000)
Oregon M	1567	18	1.15±0.269	28	2.93±0.426	2.23	3.62	0.025	0.00015
Lausanne	1155	7	0.61±0.229	13	1.73±0.384	0.73	1.57	0.46	0.115
Lausanne lead	1374	25	1.82±0.369	34	4.29±0.547	3.50	5.34	0.00015	0.000000
		(21)	(1.53±0.331)*	(31	3.78±0.514)	(3.27)	(5.07)	(0.0001)	
Period 3 (Sept.-Oct.)									
Pikes Peak 30 days									
Oregon lead	1315	14	1.06±0.282	31	3.42±0.501	1.91	4.06	0.055	0.0001
Lausanne lead	1183	15	1.27±0.326	27	3.55±0.538	2.32	4.20	0.02	0.00001
Period 4									
Madison 30 days									
Oregon controls	1432	6	0.42±0.171	10	1.12±0.278	—	—	—	—
Oregon lead	1504	12	0.80±0.230	14	1.73±0.336	1.33	1.49	0.185	0.16
		(9	0.60±0.199)*	(11	1.33±0.295)	(0.87)	(0.79)	(0.38)	(0.42)
Lausanne controls	1600	7	0.44±0.166	11	1.13±0.264	—	—	—	—
Lausanne lead	1595	8	0.50±0.177	15	1.44±0.298	0.25	0.78	0.80	0.425

* Corrected figures in parentheses.

† Diff./S.E. Diff.

The results are, in general, very similar in both stocks. It should be mentioned that the mutation rates in the controls from both stocks were found somewhat higher than usual: 1.71 percent and 1.75 percent (when a four to one sex-ratio is used as indicator for semilethals) instead of about 1.35 percent found for the Oregon stock in 1936 and in previous examinations, and 1.64 percent instead of 1.2 to 1.3 for the Lausanne stock. The percent of lethals found in the Lausanne stock was 0.39 ± .19 instead of

0.24 ± 0.09 percent determined by DEMEREC (1937) for the same stock. These high mutation rates were practically the same at the beginning and at the end of the experiments.

The table shows, furthermore, that exposure to the five to six fold higher frequencies of cosmic radiation at an altitude of about 14,000 feet (without lead) are followed by only slight increases in the mutation rate. Such slight increases were found in both exposed stocks and for each period of exposure, but none of these increases seems statistically significant.

The mutation rate in the offspring from all cultures which were kept at high altitude *under lead* for 25, 30, or 40 days, was found about three to four times higher than that of the controls at Madison. The figures are nearly the same for the 25 days and the 30 days exposures, and higher following exposures for 40 days. The positive results undoubtedly are statistically significant for lethals and semilethals in all series of exposures and for the lethals alone for the exposures for 40 days. The increase in lethal mutations following exposures for 25 or 30 days seems clearly significant only in the Lausanne stock but not beyond objection in the Oregon stock.

A statistically significant but somewhat smaller increase in the frequencies of lethals and semilethals was found also in the offspring from flies which were kept for 25 or 40 days on Pikes Peak in the metallic environment where exposures in 1936 had given similar positive results.

The cultures which were held under lead in Madison for 30 days, did not show a statistically significant increase in the mutation rate over that in the corresponding controls. The frequencies of lethal and semilethal sex-linked mutations are nearly the same as in the cultures which were exposed to the high altitude conditions without lead.

The same lead plates were used in the exposures of periods 2 and 3 on Pikes Peak and in those of period 4 at Madison, in order to exclude possible differences in the influence of lead of different qualities. This seemed more important than a simultaneous exposure at both altitudes, as the average intensities of cosmic radiation and the "normal" mutation rate of the stocks could be supposed to remain practically constant during the whole course of these experiments. The constancy of the mutation rate in the controls from both stocks was demonstrated later by the figures given in table 1.

The figures shown in table 1 are in general consistent in both stocks and for lethals as well as for lethals and semilethals. There seem to be only two slight differences in the results: the mutation rate in the Lausanne stock seems somewhat higher than that of the Oregon stock following the second period of exposures at high altitude under lead, and the frequencies of

lethals were found higher in the Oregon stock than in the Lausanne stock following the exposure at Madison under lead.

These differences, however, can be attributed to an inexactness in the determination of the number of *separate* mutations (see below). They disappear completely and the results become strikingly consistent in all periods of exposure and for the increases in the frequencies of lethal and those of lethal and semilethal mutations when the necessary adjustments of the determinations are made. (See table 1, "corrected figures," and table 4.)

Thus the results of all tests seem to confirm the conclusions which were drawn from the observations in 1936 and to allow the following statements:

1. The ordinary five–six fold increase in the intensities of cosmic radiation at an altitude of 14,000 feet is not sufficient to produce a statistically significant increase in the mutation rate within the limits of our separate experimental tests.

2. A further increase in the frequencies of secondary radiation by the passage of primary radiation through heavy metals, leads to a significant increase in the mutation rate.

3. There is no "seasonal" variation in the results of exposures of Drosophila stocks to the frequencies of cosmic radiation at an altitude of 14,000 feet, with or without lead. The explanation of the difference in the results of exposures for similar lengths of time in July–August and in September 1936 by an influence of the "metallic environment" on the intensities of secondary radiation during the July–August exposures holds true. Cultures which were exposed within this environment, showed again a significant increase in their mutation rate. Simultaneous exposures of sister cultures, which were kept far away from metallic surroundings, had much weaker (or doubtful) results.

None of the cultures, which were kept at the high altitude *without lead* and far away from any metallic surroundings, showed a statistically significant increase in the mutation rate. But the records show the same tendency towards an increase in the frequencies of mutations in both exposed stocks and for both periods of such exposures.

Thus it seems very probable that even an only five fold increase in the frequencies of cosmic radiation has some influence on the frequencies of mutations. But the increase in the mutation rate following exposures for 25 or 40 days was too small to show significant differences within the reach of the separate tests.

The effect of these exposures on the mutation rate becomes conspicuous when we combine the figures from the Lausanne and the Oregon stocks, and for both periods of exposures at the high altitude without lead, and correspondingly the figures for the two separate tests of the controls from

both stocks. This procedure seems fully admissible. The mutation rate of the Oregon stock and that of the Lausanne stock are nearly identical under the "ordinary" conditions at Madison, and the results of the exposures are strikingly consistent in both stocks (table 1).

The combined figures are shown in table 2. They indicate that the exposures at the high altitude without lead were followed by an increase of about 1.5 times in the mutation rate. Even these figures are not significant statistically for the lethal mutations, but they are significant when we consider the increase in lethals and semilethals. The probability of the increase being a chance result is only 0.02.

TABLE 2

Frequencies of lethal and semilethal sex-linked mutations.

CULTURES	NUMBER OF TESTED X CHROMOSOMES	LETHALS		LETHALS AND SEMILETHALS		DIFFERENCE TEST AND CONTROLS*		PROBABILITY FOR		INCREASE OVER CONTROLS	
		NUMBER	PERCENT	NUMBER	PERCENT	LETHALS	LETHALS AND SEMILETHALS	LETHALS	LETHALS AND SEMILETHALS	LETHALS	LETHALS AND SEMILETHALS
Controls	5065	21	0.41±0.090	54	1.07±0.145	—	—	—	—	—	—
Exposed at Madison under lead	3099	20	0.65±0.144	49	1.58±0.224	1.41	1.92	0.16	0.055	1.59	1.48
Exposed at high altitude	4457	28	0.63±0.119	72	1.62±0.189	1.48	2.31	0.14	0.02	1.54	1.51
Exposed at high altitude under lead Periods:											
I	2408	28	1.16±0.218	76	3.16±0.357	3.18	5.43	0.001		2.83	2.95
II	2758	47	1.70±0.246	113	4.10±0.378	4.92	7.50	0.000001	Far below	4.15	3.83
III	2498	29	1.16±0.214	87	3.48±0.367	3.23	6.12	0.001	0.0000001	2.83	3.25
I & II	5166	75	1.45±0.166	189	3.66±0.261	5.50	8.69	0.000000		3.54	3.42
II & III	5256	76	1.45±0.165	200	3.80±0.264	5.53	9.10	0.000000		3.54	3.55

* Diff./S.E. Diff.

The results of exposures at Madison under lead are very similar to those of the exposures at the high altitude without lead. Again we can combine the tests of both stocks and get the figures shown in table 2. They seem to indicate an increase of 1.59 times in the mutation rate of lethals and of 1.48 times in lethals and semilethals following an exposure for 30 days; but this difference is again not significant statistically for lethals and somewhat doubtful for lethals and semilethals. The probability of dealing with a chance result is in this case 0.16 and 0.055 respectively.

The increases in the frequencies of *lethal* mutations following exposures at the high altitude under lead for 25 or 30 days were not beyond dispute in the Oregon stock (table 1). Combinations of the figures for both stocks and periods show that the results of these exposures are undoubtedly

significant not only for lethals and semilethals but also for the lethals alone (table 2).

The figures given in table 2 seem to indicate that the mutation rate becomes increased about three times over that of the controls following exposures for 25 or 30 days, and about four times following exposures for 40 days. These figures, however, need some adjustments (table 4).

THE DISTRIBUTION OF LETHAL AND SEMILETHAL SEX-LINKED MUTATIONS AND OF OTHER ALTERATIONS IN THE OFFSPRING FROM DIFFERENT EXPOSED MALES

Table 1 and the records of the experiments of the previous year (JOLLOS 1937) show the frequencies of lethal and semilethal sex-linked mutations in each series of exposed or control flies; but they do not show the distribution of these mutations in the offspring of different males of the same series. Records of the tests were, in general, not kept separately for each exposed or control male in 1936 and for the first period of exposure in 1937. The comparatively small number of exposed males with normal fertility in the experiments performed in 1936 made it advisable to combine more than one exposed male simultaneously with a number of virgin ClB females in most of the test matings, in order to guarantee the production of a large F_1 generation. However, occasional observations in 1936, and the results of a number of tests of single exposed males from the first period of exposure in 1937, seemed to indicate that the distribution of sex-linked mutations as well as of other observed alterations, (especially of nondisjunction of the X chromosomes), was somewhat irregular in the offspring of different exposed males from the same series of exposure.

The greater number of fertile males from periods 2, 3 and 4 allowed to mate each of these exposed or control males separately with one or more virgin ClB females. The results of these tests were recorded for each male separately, as long as the mating of a tested male with virgin ClB females produced a minimum of 12 Bar-eyed F_1 females.

Table 3 summarizes the results of these separate tests. It shows clearly that the frequencies of sex-linked lethals or semilethals indeed vary significantly in the offspring from different males which were kept under the same conditions. We could roughly distinguish three groups of males: those which show no lethals or semilethals, those with one or two, and those with three or more than three lethal or semilethal sex-linked mutations in their offspring. More than 75 percent of the control males from both stocks show no lethals or semilethals in their offspring. This percentage becomes somewhat smaller in the males which were kept under lead in Madison for 30 days, (70 percent and 72 percent). It decreases further in males which were exposed on top of Pikes Peak without lead or other heavy

TABLE 3

Distribution of lethal or semilethal sex-linked mutations, of non-disjunction and "larval mortality".

STOCK AND PERIOD	NUMBER OF TESTED MALES	NUMBER OF MALES SHOWING LETHALS OR SEMILETHALS IN THEIR OFFSPRING										
		0		1	2	3	4	5	6	7	NON-DISJUNCTION	LARVAL MORTALITY
		NO.	%									
Period 2 (Pikes Peak 40 days)												
Oregon	35	22	62.86	8	4	—	1 Nd	—	—	—	1	—
Oregon lead	54	27	50.00	12	7	3 1 Nd LM 1 LM	4 3 Nd LM	—	1 Nd LM	—	5	6 (5+1)*
Oregon M	58	35	60.34	15	1	2 1 Nd	4 4 Nd LM	—	—	1 Nd LM	6	5 (5)
Lausanne	53	35	66.04	17	1	—	—	—	—	—	—	—
Lausanne lead	57	29	50.88	14 1 LM	7 1 Nd	2 1 Nd LM 1 Nd	1 Nd LM	1 Nd LM	3 2 Nd LM	1 Nd LM	7	6 (5+1)
Period 3 (Pikes Peak 30 days)												
Oregon lead	58	32	55.17	15	7	3 2 Nd LM	—	—	—	1 Nd LM	3	3 (3)
Lausanne lead	50	30 1 Nd 2 LM	60.00	12	4	—	1	1 Nd LM	1 Nd LM	1 Nd LM	4	5 (3+2)
Period 4 (Madison 30 days)												
Oregon lead	58	42	72.41	11	3 1 Nd LM	—	1	1 Nd	—	—	2	1 (1)
Lausanne lead	50	35	70.00	10	3	2 2 LM	—	—	—	—	—	2
Controls												
Oregon	58	44	75.86	13	—	1 Nd	—	—	—	—	1	—
Lausanne	70	54	77.14	15	1	—	—	—	—	—	—	—

* First number in parentheses shows the males identical with those listed under "non-disjunction."
Nd = non-disjunction, LM = larval mortality.

metals (62.86 percent and 66.04 percent). The percentage drops to 55.60 following the exposures on Pikes Peak under lead for 30 days, and to 50 in males which were exposed correspondingly for 40 days.

On the other hand, males which give more than three lethals or semi-lethals in the ClB tests are extremely rare in the controls as well as in the cultures which were kept at Madison under lead, or on top of Pikes Peak without lead; but they represented up to 10 percent of the tested males from the cultures which were exposed at the high altitude under lead or in the metallic environment.

The increase in the mutation rate is not the only kind of alteration which was observed in the offspring of exposed males. Some results of the 1936 experiments lead to the suspicion that the exposure to the higher frequencies of cosmic radiation at the high altitude in metallic environment may cause also non-disjunction of the X chromosomes. The observations in 1936 were not conclusive in this respect, as males with genetically marked X chromosomes had been used as mates of Bar-eyed F_1 females, in only a small percentage of the tests.

It seemed necessary to get more data on the frequencies of non-disjunction in the offspring of exposed and of control males from our stocks. Thus males which carried easily visible sex-linked mutations, mainly the combinations yellow-curlex-ruby, or yellow-vermilion, or the mutation "white," were mated with most of the Bar-eyed F_1 females (from crosses of test males with virgin ClB females) in the tests made in 1937.

The results are shown in table 3. Only those cases were recorded as "non-disjunctional" in which a minimum of 30 percent of the produced males carried the sex-linked inherited character (or characters) of their father. Thus no case of occasional crossing over in the X chromosomes of the Bar-eyed mother could have been recorded erroneously as non-disjunction.

The figures show that in both stocks non-disjunction was nearly absent in the offspring of control males. It occurred very rarely in the offspring of males which were kept at Madison under 18 mm lead, or at the high altitude without lead; but it was found comparatively frequently in the offspring of males which had been exposed on Pikes Peak or Mount Evans under lead, or on Pikes Peak in the metallic environment.

The tests showed, furthermore, that the exposures on the high altitude under lead or in the metallic environment sometimes were followed also by another alteration. Matings of Bar-eyed F_1 females with untreated males in several cases produced exclusively eggs which developed only to larval or pupal stages of normal appearance, but did not reach the adult stage. The background of this phenomenon is not known. It could hardly depend on genic alterations, as this "larval mortality" was found in the F_2 genera-

tion from exposed males. A connection with disturbances of the meiotic divisions seems more probable, especially since cases of this larval mortality were found mainly in the offspring of males which showed also cases of non-disjunction (table 3). No definite explanation can be given at present.

The study of the distribution of non-disjunction of the X chromosomes and of "larval mortality" reveals the fact that both types of alterations are found in the offspring of only a small minority of the tested males, that is, almost exclusively in the offspring of males which showed also several lethal or semilethal sex-linked mutations (table 3).

The coincidence between the appearance of several mutations, and of cases of non-disjunction of the X chromosomes or of larval mortality, is striking. The records demonstrate that cases of non-disjunction or of larval mortality, are found regularly and almost exclusively in the offspring of males which show at least three lethal or semilethal mutations and vice versa. There were only a few exceptions to this rule; even rarer ones than the figures in table 3 seem to indicate. The number of lethal or semilethal mutations probably were higher than recorded in some of these apparently exceptional cases. However, several probable lethals or semilethals were not taken into account as the observed sex ratios of more than five females to one male were found in offspring which were less numerous than the required minimum of 30 flies.

The high percentage of "non-disjunction" males in all cases in table 3 suggests that we are dealing here with secondary non-disjunction. Therefore one has to assume that the alterations which caused the primary non-disjunctions took place in spermatocytes or spermatogonial cells of the exposed males. Alterations in an early stage of spermatogenesis would explain also the regular occurrence of *several* X chromosomes with lethals or semilethals in the same males. The coincidence of non-disjunction, larval mortality and of several X chromosomes with lethal or semilethal mutations in the offspring of the same male indicates that more than one spermatogonium or spermatocyte must have been altered in these cases.

These observations and conclusions are of primary importance for a reliable determination of the number of separate mutations under various tested conditions. The data shown in table 1 do not distinguish alterations at an early stage of spermatogenesis. The figures shown in table 3 make it highly probable that such alterations occurred comparatively frequently in males which were exposed at the high altitude under lead. This means that the figures in table 1 are too high, at least for these series of exposures, as the same mutation has been counted several times. The necessary adjustment of the figures could be made exactly by determining the identity or non-identity of the lethal or semilethal mutations which are found in the offspring of the same male.

There was, unfortunately, no technical possibility to make localization tests simultaneously with the handling of the very numerous cultures involved in these experiments. Thus only a tentative adjustment can be made by considering several mutations as only one in all cases in which more than three lethals, or more than three semilethals were found in the offspring of the same male. Such "conservative" adjustment eliminates the few slight differences in the results from both exposed stocks. The adjusted figures are shown in table 4.

TABLE 4

Frequencies of lethal and semilethal sex-linked mutations. (Corrected figures.)

CULTURES	NUMBER OF TESTED X CHROMOSOMES	LETHALS		LETHALS AND SEMILETHALS		DIFF./S.E. DIFF. BETWEEN EXPOSED AND CONTROL CULTURES		PROBABILITY FOR		INCREASE OVER MUTATION-RATE OF CONTROLS	
		NUMBER	PERCENT	NUMBER	PERCENT	LETHALS	LETHALS SEMILETHALS	LETHALS	L+SL	L	L+SL
										TIMES	
Controls	5065	21	0.41±0.090	54	1.07±0.145	—	—	—	—	—	—
Exposed at Madison under lead	3099	17	0.55±0.133	46	1.48±0.217	0.88	1.57	0.38	0.11	1.34	1.38
Exposed at high altitude	4457	28	0.63±0.119	72	1.62±0.189	1.48	2.31	0.14	0.02	1.54	1.51
Exposed at high altitude under lead											
Period II	2758	40	1.45±0.227	103	3.73±0.361	4.24	6.84	0.00017		3.54	3.49
Period III	2498	29	1.16±0.214	78	3.12±0.348	3.23	5.45	0.001	far below	2.83	2.92
Periods II & III	5256	69	1.31±0.157	181	3.44±0.251	4.97	8.17	0.000001	0.0000001	3.20	3.22
Periods I, II, III	7664	97	1.27±0.128	257	3.35±0.205	5.51	9.08	0.000000		3.10	3.13

DISCUSSION

The main problem of these investigations was to find out whether a relation could be established between intensities of cosmic radiation and frequencies of "spontaneous" mutations. Previous experiments seemed to indicate that exposures of Drosophila stocks to the higher frequencies of cosmic radiation at an altitude of about 14,000 feet could produce significant increases in their mutation rates, if such exposures were made in a metallic environment which favors the production of secondary radiation. The "ordinary" five to six fold increase in the intensities of cosmic radiation at an altitude of 14,000 feet over those at Madison altitude seemed to be insufficient to cause a significant increase in the mutation rate.

The much more numerous tests which are reported in this paper confirm the previous conclusions. All cultures which were exposed on Pikes Peak or Mount Evans under 18 mm of lead for periods of 25 to 40 days showed a significantly higher mutation rate than the controls at Madison. Ex-

posures for 25 or 30 days produced about a three-fold higher mutation rate. Exposure for 40 days increased the mutation rate about 3.5 times (table 4). Significant but somewhat smaller increases in the mutations rate were found also following exposures on top of Pikes Peak in the "metallic environment," which seemed to be effective in the exposures made in 1936 (table 1). There is no "seasonal" variation in the results.

Exposures at the high altitude without lead and far away from metallic surroundings were followed by much smaller increases in the frequencies of mutations. These increases seemed to be insignificant within the range of each separate test; but a combination of the figures for both stocks and periods of exposure shows the significance of the increase in lethals and semilethals. The mutation rate became increased about 1.5 times over that of the controls (tables 2 and 4). The corresponding increase in lethals alone seems not significant also within the range of the combined figures.

A combination of the figures for both stocks seemed to show a similar increase in the frequencies of lethal and semilethal mutations following exposures at Madison under lead. The significance of this increase was not beyond objection but very probable; the probability of a chance result being 0.055 (table 2). The original figures for these exposures, however, had to be adjusted; and the increase in the mutation rate becomes even less significant when we consider the corrected results shown in table 4.

RAJEVSKY, KREBS and ZICKLER (1936) have reported a relatively high increase in the frequencies of mutations in cultures of a fungus which were kept similarly under lead at a low altitude (Frankfurt, Germany). Our results not to confirm their conclusions.

Thus the data from our tests may be open to objection in some parts. Nevertheless they seem to provide an approach to the determination of the quantitative relation existing between the intensities of secondary cosmic radiation and the frequencies of "spontaneous mutations" in *Drosophila melanogaster*.

The mutation rates of two pure inbred stocks of different origin were determined following exposures to four different intensities of cosmic radiation:

A. At Madison without lead.
B. At Madison under 18 mm of lead.
C. At an altitude of about 14,000 feet without lead.
D. At the same altitude under 18 mm of lead.

Physical records show that the frequencies of cosmic radiation under the conditions indicated above at C are about 5 times higher than those under the conditions A. The frequencies under D are roughly estimated as about 15 times higher than those under A, and the frequencies under B must be estimated correspondingly as about three times higher than those under A.

The corresponding figures for these different intensities of cosmic radiation and the mutation rates are shown in table 5. The frequencies of cosmic radiation at Madison under "ordinary" conditions are considered as the unit for the various frequencies of cosmic radiation. The figures for the mutation rates combine the results of the tests of both stocks and of period 1 and 2 of the exposures at the high altitude without lead and of periods 2 and 3 at the high altitude under lead.

TABLE 5

Relation between frequencies of cosmic radiation and frequencies of mutations.

EXPOSURE	FREQUENCIES OF COSMIC RADIATION	MUTATION RATE		INCREASE (OVER MUTATION RATE OF CONTROLS)	
		LETHALS	LETHALS AND SEMILETHALS	LETHALS	LETHALS AND SEMILETHALS
		PERCENT		TIMES	
Madison (controls)	1	0.41	1.07	—	—
Madison under 18 mm of lead	3	0.65 (0.55)*	1.58 (1.48)	1.59 (1.34)	1.48 (1.38)
High altitude	5	0.63	1.62	1.54	1.51
High altitude under 18 mm of lead (Periods II & III)	15	1.45 (1.31)	3.80 (3.44)	3.54 (3.20)	3.55 (3.22)

* Corrected figures are given in parentheses.

The difference between the mutation rate at an altitude of 14,000 feet under lead and that at Madison without lead was found to be statistically significant. Thus the figures for these two exposures furnish a basis for a calculation of the percentage of "spontaneous" mutation which could be related to the influence of cosmic radiation. This percentage can be found from the simple equations:

$$\left.\begin{aligned} x+y&=0.41\\ 15x+y&=1.31\end{aligned}\right\}\ \text{for lethals (corrected figures)} \qquad \left.\begin{aligned} x+y&=1.07\\ 15x+y&=3.44\end{aligned}\right\}\ \text{for lethals and semilethals (corrected figures)}$$

where x is the percentage of mutations which are produced by the influence of cosmic radiation under the ordinary conditions at Madison, and y the percentage of mutations which are not related to this influence. x and y in these equations have the values in percent $x=0.064$, $y=0.346$ for lethals and $x=0.169$, $y=0.901$ for lethals and semilethals.

This means that 15.61 (or 15.79 percent)[3] of the "spontaneous" muta-

[3] The first percentage is based on the figures and calculations for lethals, the second on those for lethals and semilethals. The practically identical results show again the striking consistency in the determinations of the frequencies of both types of sex-linked mutations in our experiments.

tions which were found in the Oregon and the Lausanne stocks under the ordinary conditions at Madison could be related to the influence of cosmic radiation, provided that the mutation rate increases in a straight line, in proportion to the increase in the intensities of cosmic radiation. The figure 15.61 $(15.79)^3$ can be considered only as the *upper limit* of this percentage as long as such proportional growth remains to be demonstrated.

The tests of the mutation rates under the influence of the intensities 3 and 5 of cosmic radiation (Madison-lead and high altitude without lead respectively) are of some help for such a demonstration. One would expect a mutation rate of $3x+y=0.54$ percent for lethals and 1.41 percent for lethals and semilethals at Madison under lead, as the values for y are supposed to be 0.346 and 0.901 respectively in all series of exposures. The actually determined mutation rates in percent were 0.55 ± 0.138 and 1.48 ± 0.217.

The expected mutation rates at the high altitude without lead are (in percent) $5x+y=0.67$ for lethals, and 1.75 for lethals and semilethals. The mutation rates in the tests were 0.63 ± 0.119 and 1.62 ± 0.189 respectively. Thus the available experimental results agree with the assumption of an increase in the mutation rate in a straight line in proportion to the increase in the frequencies of cosmic radiation from 1 to 15. More points ought to be fixed on the line which relates cosmic radiation frequencies and mutation rates, before the assumption could be considered to be proved definitely.

Further tests could also use as starting point other frequencies of cosmic radiation than those at Madison, since a general formula for the relation between intensities of cosmic radiation and mutation rate can be derived from our data.

The records show that exposures to intensities of cosmic radiation 15 times higher than those at Madison (intensity 1) produce an increase of 3.2 times in the mutation rate over that at Madison, that is, an increase of 14 units in cosmic radiation corresponds to an increase of 2.2 units in mutation rate. Therefore the slope of the line which relates mutation rate to intensities of cosmic radiation is as $2.2:14 (=0.157)$. The equation of this assumed straight line: increase in mutation rate is $0.843+(0.157 \times$ increase in cosmic radiation intensities).

It must however be emphasized that this formula is not yet sufficiently reliable, as the figures on which our calculations were based are still not exact enough, whether considered from the physical or the biological side.

The determinations of the mutation rate under the influence of various intensities of cosmic radiation need further improvement.

The occurrence of frequent non-disjunction and of up to seven muta-

tions in the offspring of the same male (table 3) indicate that alterations of spermatogonia or spermatocytes took place in several cases. Therefore the identity or non-identity of the mutations found in the offspring of the same male should be tested in order to eliminate the possibility of repeated registration of the same mutation.[4] Such tests were not made. Thus an exact adjustment of the figures shown in tables 1 and 2 was not possible; but it seems justified and necessary to correct these figures at least in a "conservative" manner by considering several recorded mutations as due to a single alteration at an early stage in spermatogenesis in all cases in which a minimum of four lethals or of four semilethals was found in the offspring of the same male. The figures used in our previous calculations are based on such adjustment. It is certainly not beyond dispute: it could have eliminated a few separate mutations; but it seems more probable that it did not go far enough to eliminate all repeated registrations of the same mutations. Thus it seems necessary to determine also the upper and the lower limits of the frequencies of mutations in our experiments.

The upper limit is shown by the not adjusted figures in tables 1 and 2. Calculations on the basis of these figures show that the upper limit of the percentage of "spontaneous" mutations at Madison altitude which could be related to cosmic radiation would be 18.12 percent (instead of 15.61 percent found on the basis of the adjusted figures.)

The minimum mutation rate is found, when not more than one lethal and one semilethal mutation in the offspring of the same male are considered as separate mutations. This lower limit of the mutation rate (in percent) was for lethals: 0.40 in the controls and 0.95 following exposures at high altitude under lead (Periods 2 and 3). The figures for lethals and semilethals are 1.05 and 2.55 respectively.

The lowest figure for the upper limit of the percentage of spontaneous mutations at Madison altitude which are related to the influence of cosmic radiation is accordingly 9.75. (It could be even somewhat lower, as also a recorded "lethal" mutation might have been identical, occasionally, with a semilethal found in the offspring of the same male.)

The true figure lies between 9.75 and 18.12 probably much closer to 9.75, provided the frequencies of secondary cosmic radiation at the altitude of 14,000 feet under 18 mm of lead were estimated correctly. Future tests can determine this figure exactly, as far as the biological determinations are concerned.

The chance of getting exact basic figures for our calculations seems more

[4] The high increase in the mutation rate which was found in 1936 following exposures on top of Pikes Peak in the "metallic environment" might be due to a great extent, to such repeated registrations of the same mutations.

remote on the physical side. The physicist is not yet in a position to determine exactly the total amount of the various secondary radiations which are produced by the passage of cosmic rays through 18 mm of lead, and which could penetrate to the genes of the germ cells of Drosophila. Thus the assumption of a 15 fold increase in the frequencies of secondary radiation on Pikes Peak or Mount Evans under lead over those at Madison without lead, is at present only a rough estimate on the basis of available partial measurements. It seems quite possible that the figure 15 has to be replaced in future by another one between 10 and 25, or even by a higher one. The upper limit of the percentage of "spontaneous" mutations, which are related to cosmic radiation under the "ordinary" conditions at Madison altitude, would be accordingly much higher or much lower than calculated before. The true figure could lie between 28.23 and 5.75 or even less (instead of between 18.12 and 9.75, table 6), probably much closer to 5.75.

TABLE 6

Upper limit of the percentage of the "spontaneous" mutations at Madison altitude which could be related to the influence of cosmic radiation.

DETERMINATION OF THE MUTATION RATES BASED ON:	ESTIMATED FREQUENCIES OF SECONDARY COSMIC RADIATION ON PIKES PEAK UNDER LEAD (SEE TEXT)		
	15	10	25
Adjusted figures	15.61	24.39	9.02
Maximum	18.12	28.29	10.56
Minimum	9.75	15.25	5.75

Thus our tests and calculations can be considered only as a rough first approach to a determination of the quantitative relation between intensities of cosmic radiation and frequencies of "spontaneous" mutations. However, they seem to show a way in which such a determination could gradually become more exact.

Our present figures, inexact as they may be, permit the definite conclusion that cosmic radiation under ordinary conditions cannot be considered as a major source of mutations. Not more than about 28 percent but probably much less than 15.61 percent of the spontaneous mutations in our stocks at Madison altitude could be related to the influence of cosmic radiation. Nevertheless the results seem to be of some general interest, even if our lowest figure (5.75 percent) should be replaced in the future by one which is a half or a third as large. They indicate, at least for a small percentage of the "spontaneous" mutations, their dependence on a definite external factor and not only on unknown intrinsic conditions of the genes.

SUMMARY

1. The mutation rate of two pure inbred stocks of *Drosophila melanogaster* was tested following exposures to various intensities of cosmic radiation for periods of 25, 30 and 40 days.

2. Exposures at an altitude of about 14,000 feet under 18 mm of lead produced an increase in the mutation rate of about 3–3.5 times that at Madison altitude without lead. Exposures at the high altitude without lead, and in Madison under lead increased the mutation rate about 1.54 and 1.34 times respectively. The results are consistent in both stocks and for lethals and lethal and semilethal mutations. The increase in the mutation rate is statistically significant only following exposures at the high altitude under lead or without lead.

The frequencies of cosmic radiation in these exposures are estimated to be about 15, 5 and 3 times higher respectively than those at Madison without lead.

3. The figures for the exposures at the high altitude under lead and at Madison without lead indicate that not more than 9.75–18.12 percent of the "spontaneous" mutations at Madison altitude could be related to the influence of cosmic radiation. It seems probable that this upper limit is closer to 9.75 percent or even below this figure. (See paragraph 5.)

A general formula of the quantitative relation between intensities of cosmic radiation and frequencies of mutation can be derived under the assumption that the mutation rate increases in a straight line in proportion to the increase in cosmic radiation frequencies.

4. The results of the exposures at the high altitude without lead and at Madison under lead agree with this assumption, but they are not yet conclusive.

5. The tests and calculations are only a rough first approach to a determination of the quantitative relation between cosmic radiation intensities and the frequencies of mutations. The basic figures in the calculations are not yet sufficiently reliable, whether considered from the physical or the biological side. The range of possible error is taken into account in the figures given in paragraph 3, as far as the biological measurements are concerned. The inexact determination of the frequencies of secondary radiation at the high altitude under lead widens this range significantly.

Necessary improvements of the biological measurements are suggested.

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A STUDY OF THE EFFECT ON DEVELOPMENT OF "MINUTE" MUTATIONS IN DROSOPHILA MELANOGASTER[1]

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Received October 27, 1938

INTRODUCTION

THE study of genetic factors influencing developmental rates has come to assume primary importance in the analysis of gene action since the discovery in several genera that the expression of an adult character is dependent upon a balanced interaction between different growth processes, the character being altered when the relative rates of the processes are changed by genetic or environmental influences. The work of GOLDSCHMIDT on Lymantria and Drosophila (1920, 1935, 1937), of FORD and HUXLEY on Gammarus (1927), of PLUNKETT on Drosophila (1926) and of KÜHN on Ephestia (1936 and earlier papers) has thus served to turn the attention of geneticists from the empirical study of phenotypes to the experimental study of gene action with the aid of genetical and environmental factors influencing gene expression through their effect on developmental rates.

In *Drosophila melanogaster* the mutations known as "Minutes" constitute a series of genetical factors modifying the rate of development. Since the discovery of the first Minute by BRIDGES in 1919 (BRIDGES and MORGAN 1923), many others have been found at a number of loci in all four chromosomes. Several Minutes are known to be deficiencies: *Ml*, *M3o*, *M*IV, *Mw*, *M*Blond, *M33a*, *M33j*, *M(2)vg*[11] and Haplo-IV; whether the others are deficiencies is not known. All have similar phenotypic effects in reducing size of bristles; most of those studied roughen the eyes, lower viability and fertility and retard development. All are dominant and lethal when homozygous.[2] These effects have been attributed by SCHULTZ (1929) to a common "Minute reaction." In addition, certain Minutes (*Mn*, *Mw*, *My*, *M*Blond, *M33j* and *Mβ*) have been found by STERN (1936) to increase the frequency of somatic crossing over; others have not been studied from this point of view.

[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University.

[2] Several recessive factors which reduce bristle size (minute-like, morula, chaetelle, bobbed, tiny bristle) are sometimes referred to as Minutes. STERN (1936) has reported that bobbed and tiny bristle produce a "physiological Minute condition" which increases the frequency of somatic crossing over. Minute-like (MOHR 1925) has phenotypic effects similar to those of the dominant Minutes but is not lethal when homozygous. Little else is known about these recessive factors which would justify their classification as Minutes, and as the Minutes are by definition dominant (BRIDGES and MORGAN 1923), the recessives will be omitted from the present discussion.

In a genetical analysis of the Minute reaction, SCHULTZ found that when two or more different Minutes are present in heterozygous condition in the same fly, the combination is not lethal, nor is there a cumulative effect on viability, fertility or developmental rate. On the basis of such tests of 16 Minutes in 55 combinations, he concluded that the primary reactions causing the Minute characters are different in different Minutes. Examination of the interaction of Minutes with Delta and Jammed showed that all the Minutes studied produce a similar effect, but with differences in intensity roughly proportional to the differences in viability, bristle reduction and duration of the developmental period. SCHULTZ therefore postulated a secondary reaction common to all Minutes, the "Minute reaction." He advanced the hypothesis that numerous growth processes occur, some of which are controlled by the wild type alleles of the Minutes. A heterozygous Minute factor causes a decrease in the rate of one of these processes, which then becomes a limiting reaction, while in the homozygous individual the reaction does not take place at all.

A study of the effect of Minutes on development was made by DUNN and COYNE (1935) and DUNN and MOSSIGE (1937), who found that the delay in eclosion of Minute flies, first estimated by BRIDGES and MORGAN (1923) and later roughly timed by SCHULTZ (1929), is chiefly due to a retardation in the egg-larval period. DUNN and COYNE (1935 and unpublished) found that, in combination with Lobe or Bar, *Mw*, *M33j* and *Ml*^{2} bring about a decrease in size of the eye; in the case of Lobe, the decrease is roughly proportional to the delay in development characteristic of the Minute used. However, in crowded cultures of Lobe and Lobe Minute individuals, they found that the delay in development brought about by the environmental factor of crowding results not in a decrease but in an increase in the size of the eye. They therefore concluded that the Minutes do not act through a simple slowing of the whole developmental process, but through a change in rate of development at some point in the egg-larval stage. According to this hypothesis, the effect of Minutes on viability and on characters sensitive to growth rate would be less when the previous development was slow, greater when the previous development was rapid. This is compatible with the effects of crowding on Lobe and with the facts that Minutes survive better at low temperatures and that the male, with a lower growth rate than the female, is less adversely affected as to viability.

The following investigation was undertaken in an attempt to discover how the Minutes prolong development, an effect which is of direct interest in understanding the action of these factors and of the processes which they influence, and as indirect evidence bearing on the action of the other genes whose expression is altered.

MATERIALS

As experimental material, Minute-w (*Mw*, III-80) and Minute-l^2 (*Ml*^{2}, II-101) were selected because, of the Minutes which have been studied in any detail, they show the most and the least extreme effect, on development respectively. Minute Florida (*MFla*), found by J. COYNE, April 1935, and located by BRYSON (1937) at III-80±, has been used for comparative purposes. *MFla* may be an allele of *Mw*. Heterozygous *MFla* flies show a delay in emergence similar to that of *Mw*.

The Columbia University stock of Florida wild type was used, inbred by brother-sister matings for 21 generations before the beginning of the investigation. Stocks of heterozygous *Mw*, *Ml*^{2} and *MFla* on a Florida background were maintained by repeated backcrosses of Minute males to Florida females. In certain experiments, use was made of a stock homozygous for claret on a background of Florida, isogenic with the Minute and wild type stocks.

All experiments were conducted at 25±0.5°C.

EXPERIMENTAL EVIDENCE: I. BODY SIZE OF MINUTE FLIES

In a study of differences in developmental rate of Minute and wild type zygotes, it is of importance to know whether growth of the imaginal tissue has continued during the prolonged period of development, resulting in a larger fly, or whether the imaginal growth rate has been reduced, as evidenced by smaller body size of the fly.

Minute flies have been described throughout the literature as tending to be smaller than wild type flies, and it may readily be seen in many flies of Minute phenotype that the body is actually smaller than that of the non-Minute sibs. This is not evident in all the Minute flies of a culture, however, and in any event the difference is slight and could by no means be used as a basis for classification. The following series of measurements was made in order to determine whether body size in Minutes is significantly less than normal.

Tibia length was used as an index of body size. Since the first use of the leg segments as an index of size by CASTLE, CARPENTER, CLARK, MAST and BARROWS (1906), the femur or tibia have been found to give reliable indication of body size differences of flies raised at different temperatures (ALPATOV and PEARL 1929; IMAI 1933, 1937), of the size differences between species (DOBZHANSKY, working with *Drosophila miranda* and *D. pseudoobscura*, 1935), and of the pleiotropic effects of certain genes (DOBZHANSKY 1930, using Stubble and stubbloid; COMBS 1937, using the Bar alleles).

A mass mating of ten pairs of flies was made for each of the following crosses: $Mw/+\,♂ \times +\,♀$, $+\,♂ \times Mw/+\,♀$, $Ml^2/+\,♂ \times Ml^2/+\,♀$,

MFla/+ ♂ × *MFla*/+ ♀ and Florida wild type. From each of these crosses, larvae were cultured in shell vials (20 to a vial). At 24 to 48 hours after eclosion of the adults, the third right leg was cut off and the tibia measured with an ocular micrometer.

The results, given in table 1, show a marked sexual dimorphism in tibia length, the female tibiae being significantly longer than those of the males. This is to be expected, since the female fly is considerably larger than the male. The wild type tibiae are slightly but significantly longer than those of the Minutes. This may be taken to mean that the Minute fly is, as inspection suggests, smaller than its non-Minute sibs. The difference between Minute and wild type is less than the difference between male and female of any given type, a fact which also may be observed by inspection.

Although in each cross, the wild type sibs serve as controls for the Minute flies, measurements for comparison were made of three Florida strains which had been raised separately without inter-crossing for five or more generations. These measurements (table 1) are in close agreement with those of the wild type sibs from the Minute crosses, an indication of physiological homogeneity of the stocks.

Reciprocal crosses were made of *Mw*/+ and wild type in order to test the possibility of maternal influence of the Minute mother upon body size of the offspring. The measurements indicate that there is probably no maternal effect of *Mw* on tibia length. As the results from the Ml^2 and *MFla* crosses are in close agreement with those from the *Mw* crosses, it is probable that here too maternal influence is lacking.

This series of experiments may be taken as evidence that the mean difference in size of Minute flies, as compared with their wild type sibs, is slight but real; the Minute imagoes are the smaller. It must be concluded that the imaginal tissue of the heterozygous Minute does not grow at the same rate as that of the wild type; if this were so, the longer duration of development would result in a larger imago. The growth rate must either be slower throughout development or be lessened at some developmental stage.

II. THE STAGE IN DEVELOPMENT AT WHICH THE MINUTE EFFECT ON GROWTH IS PRODUCED

The Haplo-IV zygote, classified by BRIDGES as a Minute, (MORGAN BRIDGES and STURTEVANT 1925), was found by LI (1927) to be retarded in all three stages of development, a significant retardation occurring in the embryonic as well as in the larval and pupal periods. The data of DUNN and MOSSIGE (1937) led these investigators to conclude that the developmental delay characteristic of the heterozygous Minute was due to a

TABLE 1
Constants for length of tibia.

CROSS	PHENOTYPE	MALES				FEMALES			
		n	MEAN MM	S.D. MM	C.V. PERCENT	n	MEAN MM	S.D. MM	C.V. PERCENT
Florida (3 strains)		92	0.67±.002	0.02±.002	7.59±.81	97	0.70±.004	0.04±.003	5.25±.38
		101	0.66±.002	0.02±.001	2.67±.19	90	0.72±.002	0.02±.002	2.84±.21
		142	0.66±.002	0.03±.002	4.28±.25	120	0.72±.003	0.03±.002	3.86±.25
Mw/+♂×+♀									
	Minutes	78	0.67±.004	0.03±.002	4.07±.33	57	0.72±.003	0.02±.002	3.09±.29
	Wild type	107	0.68±.003	0.03±.002	3.82±.26	85	0.74±.002	0.02±.002	3.31±.25
			Difference=0.01±.005				Difference=0.02±.004		
+♂×*Mw*/+♀									
	Minutes	49	0.65±.003	0.02±.002	3.19±.29	51	0.69±.005	0.03±.003	4.85±.48
	Wild type	56	0.66±.003	0.03±.002	3.81±.36	60	0.72±.002	0.02±.002	2.51±.23
			Difference=0.007±.004*				Difference=0.03±.005		
MP/+×*MP*/+									
	Minutes	62	0.66±.005	0.04±.003	5.70±.51	37	0.71±.003	0.02±.002	2.90±.34
	Wild type	38	0.67±.003	0.02±.002	3.18±.36	50	0.73±.005	0.04±.002	5.28±.53
			Difference=0.01±.006				Difference=0.02±.006		
MFla/+×*MFla*/+									
	Minutes	65	0.64±.002	0.02±.002	2.94±.26	37	0.67±.004	0.03±.003	3.87±.45
	Wild type	31	0.67±.004	0.02±.003	3.01±.38	30	0.70±.003	0.01±.002	2.08±.27
			Difference=0.03±.004				Difference=0.03±.005		

* Not significant.

prolongation of the egg-larval stage, with a negligible delay, if any, in the pupal stage. An experiment with *Ml*[2] indicated that there is no delay in hatching from the egg and that the larval stage is therefore the one affected. They found that *Mw*, *M33j*, *Mz* and *Ml*[2] prolong development about as 40:33:30:12.

As no study had hitherto been made of the development of the *MFla* heterozygote, the following experiment was planned to determine how great is the effect on duration of the developmental period of this genotype, and whether there is any prolongation of the pupal period. An experiment with *Mw* was performed at the same time, to make possible a direct comparison of the developmental periods of these two genotypes, and to relate the results of this study with the seriation of effects of the Minutes studied by DUNN and MOSSIGE.

Mass matings in vials were made of about 40 newly hatched Florida females and 40 Minute males. On the fifth day after hatching of the females, when egg laying is at a maximum, the females were allowed to oviposit during one 24-hour laying period on paraffined paper spoons containing banana agar colored with molasses. Larvae were then collected from the spoons at 2-hour intervals; the age of the larvae from hatching was thus known with an error of ± 1 hour. The larvae were cultured, 30 to a dish, in 7.5 cm Petri dishes on banana agar sown with a thick yeast suspension. The culture dishes were observed at 6-hour intervals until all the larvae had pupated; at every observation, the pupae from each dish were removed to a separate shell vial, containing a few cc of agar solution for moisture supply. The pupae were then observed at 12-hour intervals and the phenotypes of the hatched flies recorded. The time from hatching from the egg to pupation was thus known with an error of ± 3 hours; the time from pupation to emergence was known with an error of ± 9 hours.

The results, summarized in table 2, show that the larval periods of the *MFla* and *Mw* heterozygotes are prolonged by about the same number of hours. The amount of prolongation, about 41 hours, is in close agreement with that reported by DUNN and MOSSIGE, who observed a delay of 1.8 days (43 hours) in *Mw* larvae. It is also evident that there is no difference between the sexes in duration of the larval period of Minute or wild type larvae; this is in agreement with the results of BONNIER (1926) for a yellow non-Minute stock and of DUNN and MOSSIGE (1937) for *Mz*, *Mw* and *Ml* stocks.

The data given in table 2 show that the pupal period of the female is significantly shorter than that of the male in both the Minute and the wild type groups, a sex difference which has been observed in non-Minute stocks by many workers, notably BONNIER (1926). A significant prolongation of the pupal period of the *MFla* and *Mw* heterozygotes of both sexes

is also shown in table 2. In both the *MFla* and the *Mw* groups, the Minute females are considerably more delayed in emergence than are the Minute males; since the wild type females emerge earlier than the males, this must mean that while the rate of pupal development is reduced in both males and females by the heterozygous Minute condition, the effect on Minute females is much greater than that on Minute males.

TABLE 2

Duration of the larval and pupal periods of MFla and Mw heterozygotes and their wild type sibs.

CROSS	PHENOTYPE	LARVAL STAGE			PUPAL STAGE		
		n	AVERAGE DURATION HOURS	RANGE HOURS	n	AVERAGE DURATION HOURS	RANGE HOURS
+♀×*MFla*/+♂							
	+♂	97	89.4±3.0	80.0–104	97	107.0±9.0	89.5–115
	+♀	93	88.9±3.0	80.0–105.5	93	95.7±9.0	80.0–115
	MFla ♂	53	131.3±3.0	111.5–156	53	119.7±9.0	105.0–136
	MFla ♀	55	128.9±3.0	111.5–153.5	53	114.5±9.0	105.0–147
+♀×*Mw*/+♂							
	+♂	65	87.2±3.0	79.5– 99.5	53	101.0±9.0	89.5–113
	+♀	58	86.6±3.0	80.0– 99.5	58	92.2±9.0	86.5–105
	Mw ♂	43	128.5±3.0	108.0–165.5	43	116.5±9.0	111.0–129
	Mw ♀	51	129.5±3.0	111.0–159.5	51	113.4±9.0	105.0–129

Prolongation of larval period:
MFla ♂ 41.9 hours
MFla ♀ 40.0 hours
Mw ♂ 41.3 hours
Mw ♀ 42.9 hours

Prolongation of pupal period:
MFla ♂ 12.7 hours
MFla ♀ 18.8 hours
Mw ♂ 16.5 hours
Mw ♀ 21.2 hours

The pupal delay observed in this experiment is very much longer than that found by DUNN and MOSSIGE for any of the Minutes which they studied, a difference in results which can probably be ascribed to the length of the interval between observations in the two series of experiments; DUNN and MOSSIGE observed at 24-hour, the present worker at 12-hour intervals. The occurrence of a significant pupal delay in *MFla* and *Mw* is in accordance with the findings of LI on Haplo-IV. The amount of delay cannot be directly compared with that found by LI because of the difference in the temperatures at which the two investigations were conducted.

Delay in hatching from the egg has been reported in only one instance in *Drosophila melanogaster*, that of Haplo-IV by LI (1927). An experiment by DUNN and MOSSIGE (1937) with Ml^2 gave an indication that there is no delay in duration of the embryonic period in this Minute. To ascertain

conclusively whether a delay in hatching from the egg is characteristic of the Minute heterozygote, the following experiment was carried out with *Mw*, *MFla* and *Ml*^{2}.

Matings were made and eggs collected as described above. Three consecutive one-hour laying periods were used; the age of the eggs was then known with an error of ±0.5 hour. Fourteen hours after oviposition, the larvae which had already hatched on the egg spoons were removed and cultured. The egg spoons were then examined at hourly intervals until 24 hours after oviposition, and at each observation all larvae were removed and cultured. Few eggs hatch after 24 hours at 25°C; larvae which hatched later than this were retrieved next day, about 36 hours after oviposition. When the flies had completed their development, the adult phenotypes were recorded.

TABLE 3

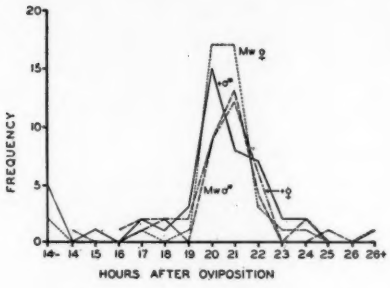
*Egg hatching time of Minute and wild type zygotes in hours from oviposition.**

CROSS	PHENOTYPE	n	M AND SE
Mw/+♂×+♀			
	Mw ♂	36	20.50±0.24
	Mw ♀	41	20.63±0.17
	+♂	41	20.49±0.27
	+♀	34	20.29±0.28
MFla/+♂×+♀			
	MFla ♂	62	20.45±0.26
	MFla ♀	44	20.57±0.13
	+♂	60	20.57±0.19
	+♀	54	20.43±0.22
Ml^2/+♂×+♀			
	Ml^2 ♂	20	19.50±0.45
	Ml^2 ♀	17	20.76±0.41
	+♂	22	19.68±0.44
	+♀	25	19.64±0.32

* In calculating the mean hatching time, individuals which hatched from the egg less than 14 hours or more than 24 hours from oviposition were disregarded.

The results, given in table 3, show conclusively that these Minutes are not delayed in hatching from the egg and that there is no sex difference in hatching time. There is only one exception to the generally consistent results, that of the *Ml*^{2} females, which hatched appreciably later than their sibs. The numbers used in the *Ml*^{2} experiment were very low, however, especially in the *Ml*^{2} female group, and it seems possible that this discrepancy is therefore not of real significance. The distribution of hatching time for *Mw* and *MFla* is shown graphically in figures 1 and 2; the curves for Minute and wild type are of the same form and are almost identical. No frequency polygon is given for *Ml*^{2} because of the small numbers involved in the experiment.

These results show that delay in hatching from the egg is not a characteristic of the Minute effect. It must be concluded that the developmental delay characteristic of the heterozygous Minute falls chiefly in the larval stage, and to a lesser extent in the pupal stage. As the delay is mainly effective in the larval stage, this period has been studied further.



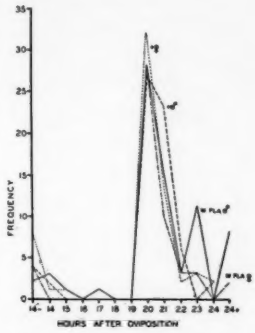


FIGURE 1 (left).—Frequency polygon of larvae hatching at successive hours in matings of *Mw*/+♂×+♀.

FIGURE 2 (right).—The same, *MFla*/+♂×+♀.

III. NATURE OF THE EFFECT ON THE LARVAL PERIOD

Number of larval instars of the heterozygous minute

It is known that the number of instars undergone during insect development, although probably under hormonal control, is not fixed in all species, and that in certain forms environmental factors may induce or inhibit moulting. One case is known in which a factor thought to be genetic induces variation in the number of instars, that of *Locusta migratoria*, where an additional moult is controlled by a "sex-linked or sex-limited factor" (KEY 1936); no breeding tests have been made in this case, however.

No departure from the usual rule of three larval instars is known in *Drosophila melanogaster*. It has been suggested (GABRITSCHEWSKY and BRIDGES 1928) that the delay of three to five days in onset of pupation in the homozygous giant larva under optimum food conditions may be associated with a fourth larval instar. The occurrence of a fourth instar has never been proven. SCHULTZ (MORGAN, BRIDGES and SCHULTZ 1936) has presented experimental evidence to show that retardation of secretion of a pupation hormone is the cause of the lengthening of the larval period in giant, but this does not exclude the possibility of the occurrence of a fourth instar during the additional larval interval. Since heterozygous *Mw* and *MFla* retard the onset of pupation for two days or more, it

might be supposed that these Minutes pass through a fourth larval instar, though such a supposition seems improbable for *Ml*^{2}, where the delay in the larval period is slight. The following technique was devised to clarify this point.

Ten eggs from a mating of *Mw*/+ male by Florida wild type female were placed in each of twelve 7.5 cm Petri dishes, containing a thin layer of Pearl's S 101 medium and sown with a thick drop of banana-grown yeast suspension. When all larvae in the cultures had pupated, the pupae were removed to paper spoons containing a small amount of agar solution (for moisture supply) and were kept in shell vials until emergence; the adult phenotypes were then recorded. After removal of the pupae, each dish was examined on a white background under the binocular; the moulted mouthparts could easily be seen on the almost transparent medium. A piece of graph paper under the dish afforded a means of orientation, so that the entire dish could be thoroughly examined. In this way it was possible to collect all moulted mouthparts. The absence of an extra set, in addition to the first and second instar sets and the third set shed in the pupa case, would prove the absence of a fourth instar. Mouthparts overlooked in this inspection were recovered by melting the medium and filtering through a small cone of filter paper, on which they were located under the binocular.

Only two sets of discarded mouth armatures were found for each larva which pupated. Since half the larvae were heterozygous Minutes it must be concluded that the heterozygous *Mw* larva passes through three instars. A similar test made with the offspring of matings of *Ml*^{2}/+ and of *MFla*/+ males by Florida females gave the same results.

The evidence is conclusive that the delay in onset of pupation of the Minutes used in this investigation is not due to an additional instar in the larval stage.

The effect of heterozygous Mw and Ml^2 *on larval growth*

Growth in the larval period has been studied for few mutant genotypes in Drosophila despite its importance in an understanding of the production of adult phenotypes. Growth of the larva as a whole has been studied only for the case of giant by GABRITSCHEWSKY and BRIDGES (1928), for vestigial by ALPATOV (1930) and for chubby by DOBZHANSKY and DUNCAN (1933); homozygous giant larvae show no change in growth rate but a delay in the onset of pupation; vestigial larvae show a decrease in growth rate as compared with their wild type sibs; chubby larvae show no change in growth but an altered ratio of length to width from the beginning of larval life.

Since in the Minutes under consideration the larval period is lengthened,

while the adult size is not greater than that of wild type, it might be expected that, were this lengthening due to changed rate of growth or smaller initial size, there would be evidence of a reduced body size of the Minute larvae at some point in development. It would be expected that a population of larvae of the same age, including Minutes and wild type sibs, would fall at some time into two size groups, giving a bimodal frequency curve of larval size. Such bimodal curves have been obtained by KERKIS (1933) for the larval length of populations of *Drosophila melanogaster*×*D. simulans* hybrids, where one sex falls behind the other in growth rate as early as 24 hours after hatching from the egg.

Length of larvae

Crosses of *Mw*/+ ♂ by + ♀, *Ml*^{2}/+ ♂ by + ♀ and Florida wild type were used to give populations which would comprise one Minute to one wild type larva, and the controls respectively. The Florida stock was in the 21st generation of brother-sister matings at the beginning of the experiment. Eggs were collected at two-hour intervals by the method described above; the age of the eggs was thus known within one hour. Age of the larvae was taken as the number of hours after oviposition. When the flies were eight days old, they were no longer used as parents; within the limits of one to eight days, however, no attempt was made to control the ages of the flies, since POWSNER (1935) has found that the duration of the egg-larval period is not different for the offspring of parents three days and seven days old.

In the case of larvae measured at 24 and 36 hours, the eggs were allowed to hatch and the larvae to develop on the spoons. In all other groups, however, the eggs were transferred to agar slants in shell vials; 20 to 22 eggs were placed in each vial. The medium was that described in the above experiment on egg-hatching time. At the desired age, the larvae were removed from the medium with a blunt needle, and were fixed in boiling water, in which they relaxed to their full length in a straight position (ALPATOV 1929). They were measured immediately with the aid of an ocular micrometer. Larval length was measured from the tip of the anterior segment to the tip of the posterior spiracles, as shown in diagram by IMAI (1937).

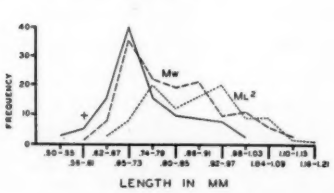
24-hour larvae. It was found in the experiments on egg hatching time that at 24 hours after oviposition almost all larvae have hatched, a few larvae are between five and ten hours old, a few are two hours old or less, while the vast majority are about four hours old, the mean hatching time falling between 19 and 20 hours. The Minute larvae hatch at the same mean time as the wild type sibs. Such a situation should result in a variable group of larvae at 24 hours, with no evidence of a bimodal curve. The fre-

quency polygon for the larvae of the three crosses at 24 hours (fig. 3) shows this situation. The biometrical constants for this age group, given below, show that the groups composed of Minute and wild type larvae are not smaller in mean length than the Florida control group.

	n	Mean (mm)	S D	C V
Florida	108	0.74±0.01	0.12±0.008	15.44±1.05%
Mw/+♂×+♀	138	0.82+0.01	0.12+0.007	14.76±0.88%
*Ml*²/+♂×+♀	102	0.88±0.01	0.11±0.008	13.25±0.93%

36-hour larvae. Only *Mw* populations of this age were measured, to test for a possible bimodal distribution of larval length; no such distribution was found.

48-hour larvae. The results of measurements of this age group are given below and in figure 4.



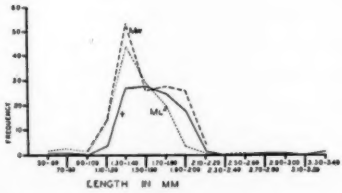


FIGURE 3 (left).—Larval length at 24 hours from oviposition in populations of heterozygous Minute and wild type larvae.

FIGURE 4 (right).—The same, 48 hours from oviposition.

	n	Mean (mm)	S D	C V
Florida	109	1.69±0.04	0.39±0.03	23.21±1.57%
Mw/+♂×+♀	155	1.54±0.02	0.30±0.02	19.29±1.09%
*Ml*²/+♂×+♀	121	1.49±0.03	0.28±0.02	18.97±1.21%

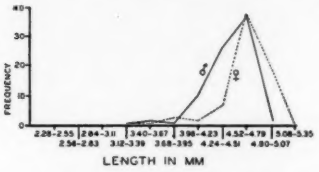
Variability is very great in all three populations, and the curve for *Mw* appears to be bimodal, results which are undoubtedly due to occurrence of the first moult at this time. The mean lengths of the Minute populations are somewhat less than that of the wild type, but in view of the high variabilities in all three cases it is inadvisable to draw conclusions concerning the growth of Minute larvae from these data.

96-hour larvae. The 96-hour and older larvae were classified for sex by observation of the gonads (KERKIS 1933) and were separated before fixation. The data given in table 4 show that in all three groups there is marked sexual dimorphism in larval length, the males having a lower mean length and lower range than the females. No data on this point have hitherto been available.

The variabilities in all groups have greatly decreased, and the frequency polygons (figs. 5, 6, 7) show no true bimodal distribution in any group.

The mean larval lengths of the Minute groups are slightly but significantly lower than that of the Florida; this is true for both sexes. These data suggest that the *Mw* and *Ml*[2] larvae are shorter than the wild type, the difference being so slight that the frequency polygons of larval length of Minute and wild type are not visibly bimodal.

100-hour and 144-hour larvae. Measurements of larvae at 100 hours were made only for the heterozygous *Mw* group, in order to determine whether



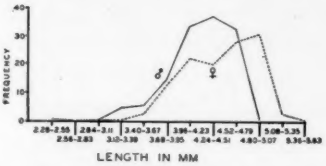
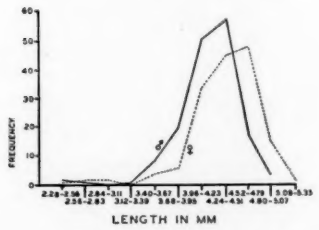


FIGURE 5 (left).—Larval length at 96 hours from oviposition, wild type.
FIGURE 6 (right).—The same, heterozygous *Mw* population.



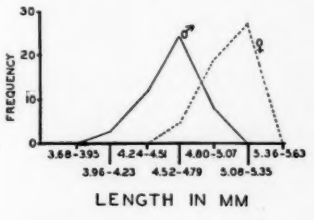


FIGURE 7 (left).—Larval length at 96 hours, heterozygous *Ml*[2] population.
FIGURE 8 (right).—Larval length at 100 hours from oviposition, heterozygous *Mw* population.

TABLE 4

Constants for larval length of wild type and heterozygous Minute populations.

HOURS AFTER OVIPOSITION	CROSS	MALES				FEMALES			
		n	MEAN MM	S.D. MM	C.V. PERCENT	n	MEAN MM	S.D. MM	C.V. PERCENT
96	Florida	179	4.43±0.03	0.29±0.02	6.43±0.51	72	4.62±0.04	0.33±0.03	7.10±0.59
	Mw/+♂×+♀	133	4.21±0.03	0.38±0.02	8.91±0.55	127	4.43±0.04	0.49±0.03	11.06±0.69
	Ml[2]/+♂×+♀	166	4.16±0.03	0.38±0.02	9.01±0.49	162	4.38±0.03	0.41±0.02	9.41±0.52
100	*Mw*/+♂×+♀								
	1st experiment (+ and Minute larvae)	53	4.36±0.04	0.27±0.03	6.24±0.61	47	4.69±0.05	0.34±0.03	7.26±0.75
	2nd experiment (+ already pupated)	46	4.35±0.02	0.15±0.02	3.51±0.37	42	4.66±0.03	0.22±0.02	4.81±0.52
144	*Mw*/+♂×+♀ (only *Mw* present)								
	1st experiment	47	4.61±0.03	0.21±0.02	4.64±0.47	51	5.06±0.02	0.17±0.02	3.31±0.33
	2nd experiment	74	4.42±0.03	0.23±0.02	5.28±0.51	56	4.94±0.03	0.19±0.02	3.78±0.36

a difference in length exists between the non-Minute larvae which are about to pupate, and the Minutes which are to continue as larvae for two days longer. Under the conditions of the experiment, pupation had begun at 100 hours; five pupae and 100 larvae were recovered from the cultures. The measurements show that sexual dimorphism in larval length at 100 hours is very marked (table 4 and fig. 8) but the curve for each sex is compact and unimodal.

At 144 hours, all wild type larvae in the heterozygous population have pupated, while the Minute larvae will begin to pupate at about 146 hours under the conditions of the experiment. The 144-hour population, therefore, is composed entirely of Minute larvae which are about to begin pupation. Measurements show that the marked sex dimorphism is maintained (table 4); the difference between the means of the male and female groups is clearly significant. Comparison of the mean larval length of the 100-hour group, composed of non-Minutes about to pupate and of Minutes which will not pupate for two more days, and of the 144-hour group of Minutes reveals a slight but significant growth in length in both sexes during the additional two days of the Minute larval period (difference = 0.256 ± 0.048 mm for the males, 0.368 ± 0.054 mm for the females).

It appears, therefore, that growth in length is not completely inhibited during the additional days of the Minute larval period. In order to be certain of this point, the 100-hour and 144-hour measurements were repeated, one year later, when the Florida stock was in the 49th inbred generation. The same technique was used as in the earlier experiment, except that the larvae were cultured in 7.5 cm Petri dishes, on two percent banana agar, sown with a thick yeast suspension; 30 larvae were grown in each dish. On this medium, pupation of the wild type occurred earlier than under the conditions of the previous experiment. At 100 hours, 48 percent of the larvae had pupated, virtually all those expected to fall within the wild type group. The 100-hour measurements, therefore, are those of heterozygous *Mw* larvae at the beginning of the two additional days of larval life. The Minute group was probably also closer to pupation than in the earlier series of measurements, but no direct test was made of this.

The results (table 4) show that between 100 and 144 hours there is a significant increase in length of the females (difference $= 0.285 \pm 0.042$ mm). The males show an increase in length which falls just short of significance (difference $= 0.076 \pm 0.038$ mm). The main conclusion of the first experiment is substantiated, that the Minutes continue to increase in length during the additional hours of larval life.

Width of larvae

The foregoing measurements were made at a time when no information

was available which would make it possible to distinguish Minute from wild type larvae. Use was later made of the observation of BEADLE (1937) that the Malpighian tubes of larvae homozygous for claret are colorless while those of wild type eye color are yellow. The offspring of matings of *Mw/ca* ♂ by *ca/ca* ♀ were cultured on two-percent banana agar in 9 cm Petri dishes, sown with yeast suspension; 40 larvae were grown in each dish, and their age was known within one half hour from hatching from the egg. The larvae were classified at 82 hours from hatching (about equal in age to the groups described above at 100 hours after oviposition; pupation of the wild type had begun), and it was then seen that the Minute larvae are smaller in diameter than the non-Minute (fig. 10). The width

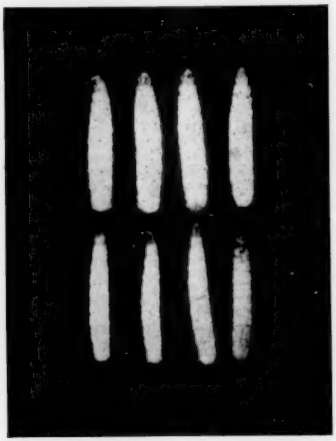


FIGURE 10.—Photograph of wild type female (upper row) and heterozygous Minute-*w* female (lower row) larvae, 82 hours from hatching from the egg.

was measured from a dorsal view, at the posterior border of the third thoracic segment and of the seventh abdominal segment respectively, with the results given in table 5.

The width of the Minute larvae at each of the levels measured is less than that of the wild type; the difference between Minute and wild type male, and between Minute and wild type female is clearly significant in each case. The Minute and non-Minute groups of the same sex show no overlapping in width of the seventh abdominal segment (fig. 9); in width of the third thoracic segment, there is some overlapping of the distributions of the two genotypes (range, for Minute males = 0.471 to 0.599 mm, non-Minute males = 0.556 to 0.685 mm; for Minute females = 0.471 to 0.642 mm, non-Minute females = 0.556 to 0.728 mm). Sexual dimorphism

TABLE 5

Size of Minute and wild type larvae at 82 hours after hatching.

	n	MEAN MM	S.D. MM	C.V. PERCENT
Width of 3rd thoracic segment:				
Wild type males	53	0.626±0.003	0.025±0.002	3.95±0.38
Mw males	66	0.546±0.003	0.024±0.002	4.46±0.39
		Difference=0.080±0.004		
Wild type females	61	0.666±0.003	0.027±0.002	4.11±0.37
Mw females	62	0.576±0.004	0.030±0.003	5.28±0.47
		Difference=0.090±0.005		
Width of 7th abdominal segment:				
Wild type males	55	0.749±0.003	0.024±0.002	3.20±0.30
Mw males	66	0.606±0.003	0.028±0.002	4.65±0.37
		Difference=0.143±0.004		
Wild type females	54	0.799±0.003	0.023±0.002	2.84±0.27
Mw females	56	0.653±0.004	0.028±0.002	4.32±0.41
		Difference=0.146±0.005		
Length of larvae:				
Wild type males	55	4.297±0.029	0.214±0.020	4.98±0.47
Mw males	74	4.234±0.016	0.144±0.012	3.40±0.27
		Difference=0.063±0.033		
Wild type females	61	4.494±0.024	0.191±0.017	4.24±0.38
Mw females	73	4.418±0.022	0.185±0.015	4.19±0.35
		Difference=0.076±0.032		

of larval width is evident and roughly corresponds to that observed above for larval length.

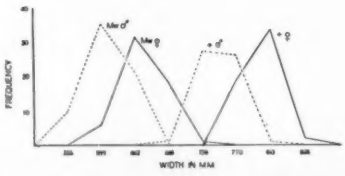


FIGURE 9.—Width of seventh abdominal segment of larvae at 82 hours after hatching from the egg, heterozygous *Mw* population.

Length of the larvae of this population was also measured, and the inference drawn from previous measurements of length, that the Minute larvae are slightly smaller than the wild type, was found to be correct (table 5). In the female group, the Minutes are slightly but significantly shorter; in the male group, the Minutes are shorter but the difference is not significant. A frequency polygon constructed from these data, with one curve for males of both genotypes and one for females of both genotypes, is very similar to that shown in figure 8 and is not bimodal.

Growth of the Minute larvae

From the data given above, it is evident that growth in length of the heterozygous Minute larvae takes place at a slightly lower rate than that of the wild type, and that after the time of puparium formation of the wild type, the Minutes continue to grow in length, until at their puparium formation they are slightly longer than the wild type prepupae. Growth in width of the larva is markedly altered, so that at the time of puparium formation of the wild type, the width of the Minute larva is less than that of the wild type. Whether there is an altered ratio of growth in length to that in width throughout larval development, or whether the initial ratio of length to width differs in the embryo from that of wild type is a question which is now under investigation.

DELAY IN OCCURRENCE OF LARVAL MOULTS

In the offspring of matings of *Mw/ca* and *MFla/ca* males by *ca/ca* females, it was observed that at 48 hours after hatching from the egg the non-Minutes were appreciably larger than their Minute sibs, and that most of the non-Minutes had undergone the second moult. Two such populations were classified for larval instars by means of the structure of the spiracles (AUERBACH 1936) and for genotype by means of Malpighian tube color, with the following results:

Population at 48 hours:

Second instar	Third instar
4 claret, 17 *Mw*	25 claret, 1 *Mw*
0 claret, 21 *MFla*	17 claret, 4 *MFla*

The second moult of the Minute larvae was evidently delayed. The following experiments were then conducted to ascertain the time at which the first and second moults take place in the Minute larvae and their wild type sibs.

Larvae from the crosses described above were collected at intervals of 2 hours from hatching from the egg, and were cultured in Petri dishes, as in section II. Their age from hatching was then known within ± one hour.

Beginning at 19 hours from the time of hatching, the larvae were observed every hour, and those which had passed into the second instar were removed and classified by Malpighian tube color. The sexes were not classified at this age, since classification of such small larvae is slow and impractical with so short an observation period and so large a population. The results (table 6) show that the heterozygous *Mw* and *MFla* larvae undergo the first moult at an average of about 26 hours after hatching, one hour later than the average hatching time of their wild type sibs. The beginning of moulting is delayed 2 hours in the case of the *MFla* heterozy-

gotes, and 3 hours in the case of *Mw* under the conditions of the experiment.

Similar observations were made from the 42nd hour of the larval period until all larvae had passed into the third instar. At this age the larvae could be classified for sex with speed and accuracy. The results (table 6) show that the *Mw* and *MFla* heterozygotes undergo the second moult at an average of about 3 hours later than their wild type sibs, while the beginning of the moult is delayed from 5 to 6 hours. The moulting periods are almost discontinuous; practically all wild type larvae have moulted when the Minutes begin to moult. There is no sex difference in time of the second moult in either Minute or wild type.

TABLE 6

Time of occurrence of the larval moults at 25°C.

HOURS FROM HATCHING	FIRST MOULT			
	CROSS: *ca/ca* ♀ × *MFla/ca* ♂		CROSS: *ca/ca* ♀ × *Mw/ca* ♂	
	CLARET	MINUTE	CLARET	MINUTE
22	6		3	
23	4		3	
24	16	8	6	
25	26	24	35	18
26	16	32	12	25
27	2	14	4	12
28		4		3
n	70	82	63	58
Average age at moult	24.6	25.7	24.9	26.0

HOURS FROM HATCHING	SECOND MOULT							
	CROSS: *ca/ca* ♀ × *MFla/ca* ♂				CROSS: *ca/ca* ♀ × *Mw/ca* ♂			
	CLARET ♂	CLARET ♀	MINUTE ♂	MINUTE ♀	CLARET ♂	CLARET ♀	MINUTE ♂	MINUTE ♀
44	8	4			3	4		
45	4	6			4	5		
46	4	6			6	7		
47	8	8			16	8		
48	4	8	2		6	8	1	
49	2	2	12	10		1	9	6
50			10	10	1	2	13	19
51			6				6	6
n	30	34	30	20	36	35	29	31
Average age at moult	46.0	46.4	49.6	49.5	46.6	46.6	49.7	50.0

It may be concluded that the heterozygous Minute larva is delayed in undergoing the first and second moults; delay at the second moult is greater than that at the first.

The high variability in larval length of the wild type, *Ml*[2] and *Mw* populations at 48 hours from oviposition (page 149) is now seen to be due to occurrence of the first larval moult at about this time. It is a matter of common observation that, upon moulting, the larva increases greatly in size, an increase which is evident in the growth curves published by ALPATOV (1929) and others. The bimodal distribution of larval length in the *Mw* population (fig. 4) is probably due to the delay in moulting which is characteristic of the *Mw* heterozygote.

PRELIMINARY STUDY OF LARVAL HISTOLOGY

From the above data on size of the Minute adult, it is evident that the Minute factors must affect the growth rate of the imaginal tissue in such a way as to produce, after a longer developmental period, an adult which is slightly smaller than the wild type. The effects of the heterozygous Minute upon the Bar and Lobe eye and upon bristles further indicate effects of the Minute factors on the imaginal tissue. Accordingly, a histological study was made of Minute and wild type larvae. As few specimens were studied (five of each genotype and sex at a given age), the following report must be regarded as preliminary, and only tentative conclusions drawn. More detailed study is now in progress.

The offspring of males heterozygous for *Mw* and claret by claret females were cultured as in the investigation of the delay in time of pupation of *Mw* and *MFla*. Larvae were collected from the egg spoons at two-hour intervals; their age was thus known within ± one hour from the time of hatching. All larvae to be compared were offspring of the same matings and were cultured together in the same dishes. Malpighian tube color was used to classify the larvae for genotype. The larvae were fixed in Kahle's fluid, imbedded in paraffin, sectioned at 7μ and stained with gentian violet and eosin. The stages investigated in this study were those at 48, 72 and 120 hours. At 48 hours, all the larvae which were studied had entered the third instar. The 72-hour larvae had passed through about two-thirds of the time required by the wild type to complete the third instar. At 120 hours, all wild type larvae had already pupated and the Minutes were about to pupate.

Larval tissue. No differences were observed at any of the stages studied between the wild type and Minute larvae in structure of the following larval tissues: hypodermis, gut and salivary glands, fat bodies, Malpighian tubes, musculature, nervous tissue. In accordance with the data given in table 5, the sections of Minute larvae at 72 hours were found to be con-

siderably smaller in diameter than the wild type (fig. 11); at 48 hours, they were smaller but the difference was not so great as at 72 hours. The larval cells of the Minute appear smaller than those of the wild type, but careful measurement of the cells must be made before this observation can be considered reliable.

Comparison was made of the organ known as Weismann's ring (WEISMANN 1864) or the ring-gland (HADORN 1937), which is the source of the pupation hormone. The gland was identified from dissections and from the descriptions of HADORN (1937) and SCHARRER and HADORN (1938). At 48 and 72 hours, the gland is conspicuous in both Minute and wild type larvae; no constant difference was found between the glands of the two genotypes. The size of the gland was variable in the specimens studied; some Minute larvae had ring-glands of the same size as the largest of the wild type glands. Whether the gland cells were actively secreting in either genotype could not be determined with the stains which were used.

Imaginal disks. This study was confined to the thoracic disks and cephalic complex; the small dorsal prothoracic disk was not studied in sufficient detail to warrant discussion here.

In the large larval cells, all nuclei observed were in the resting stage; this is in conformity with the fact reported by many investigators that mitoses are rare in the larval cells after hatching. In the imaginal disks, however, wherever one disk was larger than another, it was composed of a larger number of small cells; the differences in cell number between disks of different size were very striking, although no cell counts were made to

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FIGURE 11.—Photomicrographs of larvae in cross section, ×120. 72 hours from hatching from the egg, females.

Upper left: wild type; section through the middle region of the ventral prothoracic disk. The section is cut at an angie and shows the left ventral prothoracic and the right ventral mesothoracic disk.

Upper right: wild type; section through the middle region of the dorsal mesothoracic disk. The section is cut so that a more anterior region is shown at the right than at the left side.

Lower left: heterozygous *Mw*; section through the ventral prothoracic disks. The section is cut at an angle such that the right side is at a more anterior level.

Lower right: heterozygous *Mw*; section through the dorsal mesothoracic disk.

Key:

C G	cerebral ganglion
F	fat body
O	optic disk
OE	oesophagus
R	Weismann's ring
S	salivary gland
V P	ventral prothoracic disk
V MS	ventral mesothoracic disk
V MT	ventral metathoracic disk
D MS	dorsal mesothoracic disk
D MT	dorsal metathoracic disk

L and R used as prefixes indicate left and right.

test this quantitatively. These observations are in accordance with those of TRAGER (1935, 1937) that, in holometabolous insects, in general the tissues which are to persist into the adult stage (including the imaginal disks) grow by cell division, while those which are to be histolized in the pupal stage (most of the larval tissues) grow by increase in cell size. In the preparations used in this study, the number of mitoses in the imaginal disks could not be observed, for both division figures and plasmasomes stain heavily with gentian violet and could not be distinguished from each other.

Comparison of sections of male and female larvae showed, as would be expected from the above data on larval size, that the male tissues, including the imaginal disks, are smaller than the female. The male disks appeared in all cases, however, to have reached a stage of differentiation equal to the comparable female disks.

It was found that among the larvae of the same age, genotype and sex, the histological picture was constant; the size of the imaginal disks, especially, showed slight variability. In view of the small number of specimens, the possibility that this constancy might be due to sampling error was tested in the following way. Homozygous claret larvae, cultured in the same way as those used for histological study, were dissected in insect Ringer's solution at 72 hours after hatching, and the long axis of the right ventral prothoracic disk was measured with an ocular micrometer. The following results were obtained:

	Mean (mm)	S D	C V	n
Males	0.271 ± 0.003	0.020 ± 0.002	$7.37 \pm 0.82\%$	40
Females	0.300 ± 0.004	0.023 ± 0.003	$8.12 \pm 0.89\%$	42

These data indicate that larvae of this age are not highly variable in size of imaginal disks, and that the constancy observed in the histological preparations is probably not due to sampling error.

Ventral thoracic disks. At 48 hours, the ventral pro- and mesothoracic disks of the wild type (which are to give rise to the first and second legs and part of the thoracic hypodermis) are found in the mid-ventral region of the second and third thoracic segments, the prothoracic lying somewhat dorsal and anterior to the mesothoracic. Both pairs of disks are sac-like invaginations at this time, with the cells oriented toward the lumen of the disk; the lateral border consists of several layers of cells, the medial border of a single layer. In the heterozygous *Mw* larva at 48 hours these disks are about half as large as those of the wild type and the difference in thickness between the two cell borders of the lumen is less marked; the lumen is smaller than that of the wild type disk.

At 72 hours, the prothoracic and mesothoracic disks of the wild type

have increased greatly in size and show differentiation of the lateral cell plate into the rudiments of the segments of the legs (fig. 11, upper left). The medial cell plate has now become the peripodial membrane, one or two cells in thickness, and the lumen of the leg, into which mesenchyme cells have already migrated, has appeared at the lateral edge of the disk. In the *Mw* larva of 72 hours, the prothoracic and mesothoracic disks (fig. 11, lower left) are still sac-like, with no differentiation into leg segment rudiments and peripodial membrane. They are only slightly larger than those of the 48-hour wild type larva.

The *Mw* larva of 120 hours has progressed to an advanced stage of differentiation of the ventral thoracic disks comparable with that described by AUERBACH for the wild type larva which is ready to begin pupation. In the present investigation, no wild type larvae were studied at the time just preceding puparium formation and it is therefore impossible to tell whether the Minute imaginal tissue reaches the same stage of growth and differentiation before puparium formation as does the wild type. Comparison of the disks at 72, and 120 hours indicates, however, that during the period of prolongation of the larval stage, the *Mw* ventral thoracic disks continue to grow and differentiate.

The ventral metathoracic disks have not been studied in such detail as the pro- and mesothoracics, but it may be stated that they present essentially the same picture and are less advanced in the heterozygous *Mw* larva of 48 and 72 hours.

Dorsal thoracic disks. These disks, which are to give rise to the hypodermis of the anterior thorax and to the wing are seen in the 48-hour wild type larva as large sac-like invaginations in a dorso-lateral position in the thoracic region. The inner margin is a flat plate several cells in thickness, with the cells oriented toward the lumen of the peripodial cavity; the cavity is still small and has for its outer boundary a peripodial membrane of a single layer of cells. The disk is larger (that is, composed of more cells) than that shown in diagram by AUERBACH for a corresponding stage (her text-fig. 31). In the 48-hour *Mw* larva, the wing disk corresponds in position and structure to that of the wild type but is much smaller. At 72 hours, the wild type mesothoracic disk, in the wing-forming region (fig. 11, upper right) is composed of a large plate of cells showing the folding characteristic of the advanced third instar wing bud, a large peripodial cavity, and the peripodial membrane. The anterior, thorax-forming region of the disk is smaller and the cell plate is not folded (fig. 11, upper left). In the *Mw* 72-hour larva (fig. 11, lower left and right) the disk is much smaller in both regions than that of the wild type and in the posterior region the folding of the inner cell plate has not yet begun. At 120 hours, the Minute dorsal mesothoracic disk is in an advanced stage of

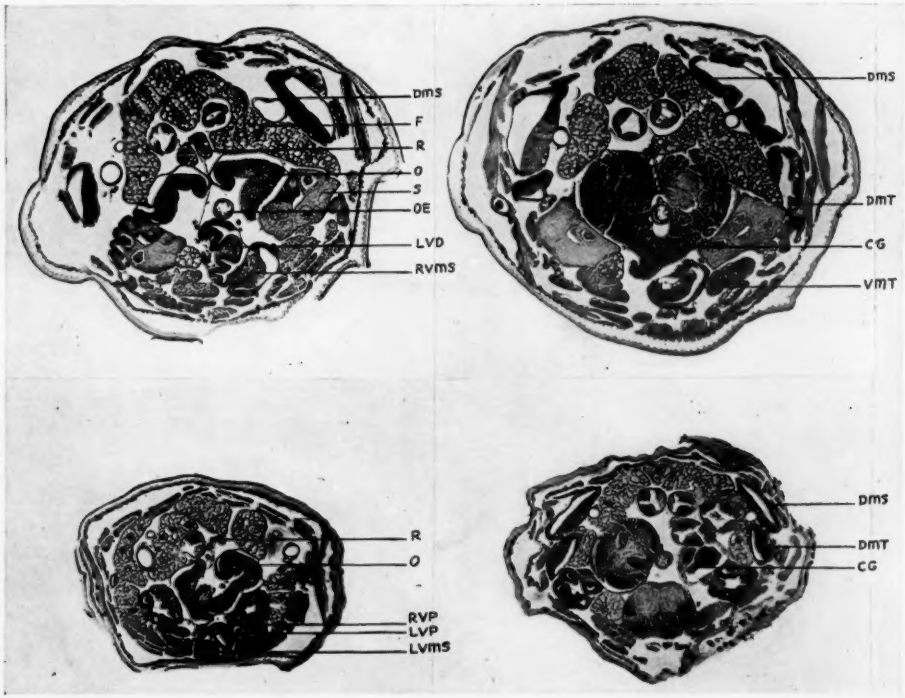

FIGURE 11

growth and differentiation, corresponding to that described by AUERBACH for the mature third instar larva of the wild type.

It may be concluded that at 48 and 72 hours the dorsal mesothoracic disk of the *Mw* larva is markedly less advanced in size and degree of differentiation than that of the wild type, and that the Minute disk continues to grow and differentiate before the larva pupates.

The dorsal metathoracic disk, which is to form the haltere, shows differences between Minute and wild type which correspond to those of the mesothoracic; these will not be described in detail.

Cephalic complex. As described by CHEN (1929), the cephalic complex is mainly composed of two pairs of imaginal disks, those of the antennae and eyes. The antennal disk is confluent with the eye disk of the same half of the complex; in the 48-hour larva, there is no sharp demarcation between the two disks of the same side but at 72 hours they are separated by a shallow transverse groove.

The antennal disk at 48 hours in the wild type is a sac-shaped structure histologically much like the ventral thoracic disks. In the *Mw* larva of the same age, the disk is smaller and less advanced as to differentiation of the cell layers; the difference between the disks of the two genotypes is comparable to the differences found in the ventral thoracic disks. At 72 hours, the picture is again similar to that of the ventral thoracics; the Minute antennal disk is much smaller; the wild type disk is well differentiated into the rudiments of segments, while this process has not begun in the cell plate of the Minute disk. The 120-hour *Mw* larva shows a well developed antennal rudiment with division of the cell plate into segments.

In the 48-hour larva of *Mw* and wild type, the optic disk is an elongate plate of cells of the typical imaginal shape, oriented toward the lumen; the lumen is present only as a slit except in occasional places where pressure seems to have separated the two cell layers of the disk; the outer layer is one or two cells thick and the cells are somewhat flattened. There is little histological difference between the *Mw* and wild type optic disk at 48 hours; the *Mw* disk, however, is considerably smaller than the wild type. The cell plate of the wild type, because of its size, has assumed a curved position around the oesophagus, while the *Mw* cell plate is still wedge-shaped.

In the 72-hour wild type larvae, the cell plate of the optic disk has retained the shape and cellular arrangement of the 48-hour disk, but has increased in cell number and, due to its greater size has assumed a curved and folded shape (fig. 11, upper left). The lumen is large and consequently the cell layer bounding the lumen on the outer side of the disk is well separated from the cell plate. In the *Mw* larvae of this age, the optic disk

is at the same stage of differentiation as the wild type, and no difference could be observed in the size of the disks of the two genotypes. This conclusion was made only after careful comparison of camera lucida drawings of corresponding cross sections of the larvae, and it is true of all the larvae studied. In the 120-hour *Mw* larva, the optic disk has increased in size and apparently in differentiation, but no detailed study was made of this.

The small number of specimens makes impossible a definite statement concerning this series of relations of the optic disks, but the evidence suggests that the optic disk of the *Mw* larva grows more slowly than the wild type during early larval development, then changes its rate of growth and catches up to the wild type by the time the 72-hour stage has been reached.

Conclusions. The histological data indicate that both increase in cell number and tissue differentiation are retarded in the antennal, ventral thoracic and dorsal meso- and metathoracic disks of the *Mw* larva as compared with those of the wild type. Although the specimens studied were not sufficiently numerous to make this conclusion final, it meets the expectation drawn from the size of the Minute fly, whose thoracic and head appendages are in fact smaller than those of the wild type. It is suggested that the optic disks of the Minute larva increase more slowly in cell number than those of the wild type in early larval development, then increase in rate of cell division and become as large as the wild type disks by 72 hours. These characteristics of growth of the optic disk are not unexpected in view of the fact that the eye of the Minute fly is not smaller than the wild type, as are the organs developing from the other disks described, but may be actually larger; and of the fact that the Minute eye is more reduced in size by the Bar and Lobe factors (DUNN and COYNE 1935) than is the wild type eye, an effect which must take place fairly early in larval development and which would be made possible by a lower growth rate of the Minute tissue at this time.

It seems probable, although further test is necessary, that the *Mw* factor affects imaginal disk development. Since the other Minutes studied in this investigation resemble *Mw* so closely in their effect on development, it is probable that this effect is not peculiar to *Mw* but is characteristic of the other Minutes also.

It should be pointed out that, in the present study, the imaginal tissues have been studied only in the larval stage, and their history during the pupal period is not known.

IV. THE FATE OF THE MINUTE HOMOZYGOTE

It has been reported by LI (1927) that the *Ml* homozygote dies in the early egg stage, but no other data on the lethal effect of the homozygous

Minute condition have hitherto been available. During the course of the present investigation, it has been found that the *Mw* and *Ml*^{2} homozygotes die during the first larval instar; and that of the *MFla* homozygotes, a small proportion die just before hatching from the egg but the vast majority die during the first instar. The homozygous Minute larvae grow only very slightly and do not moult, although they may survive for three days after hatching at 25°C. Cultures of such larvae on food mixed with borax carmine show that some larvae take in a small amount of food, while others do not feed at all.

The homozygotes may be distinguished from their wild type and heterozygous Minute sibs by their small size and sluggish, flaccid appearance, in which they resemble the *ClB* male larvae (BREHME 1937). Preliminary histological examination has revealed a deficiency of the fat bodies and a general necrotic condition of all larval tissues in the larvae surviving until 48 and 72 hours from oviposition. A full report of these observations will be published separately.

DISCUSSION

This investigation was carried on in an attempt to discover the nature of the effect by which the heterozygous Minute factor brings about a prolongation of the developmental period and the production of a smaller imago. Four differences have been found between development of the Minute and of the wild type: the time of puparium formation is delayed; there is a delay in the occurrence of the first and second moults of the Minute larva; larval growth of the Minute is affected in such a way that at the time of puparium formation of the wild type, the Minute larva is markedly narrower and slightly shorter than the wild type; and certain of the Minute imaginal disks are retarded in growth and differentiation.

The first effect, that of delay in puparium formation, may be attributed to a delayed or insufficient secretion of pupation hormone. It is also possible that the hormone is produced in normal quantity at the time when the wild type forms the puparium, but that the Minute tissues are not able at that time to react by forming a puparium. The data presented in this paper give no means of distinguishing between these three possibilities. It is established that in the holometabolous insects, the process of pupation is initiated by a hormonal stimulus. In the Diptera, an approximate location of the source of the hormone was made by FRAENKEL (1935); in *Calliphora erythrocephala*, he found the hormone to arise in the cerebral ganglion or its immediate neighborhood. In *Drosophila melanogaster*, HADORN (1937) has demonstrated by transplantation methods that the hormone is produced by the "ring-gland" or Weismann's ring, a structure suggested by BURTT (1937) to be the homolog in the Diptera of the corpus

allatum of other insects. This body is the source of the metamorphosis-inhibiting hormone in the hemimetabolous insect, Rhodnius (WIGGLESWORTH 1934).

In two cases in *Drosophila melanogaster*, delayed puparium formation has been studied from the physiological point of view. SCHULTZ (MORGAN, BRIDGES and SCHULTZ 1936) has suggested that the giant larva, delayed three to five days in pupation, pupates later because of its failure to produce a sufficient quantity of pupation hormone at the usual time. HADORN (1937), by transplanting wild type "ring-glands" into homozygous lethal-giant larvae, has obtained an acceleration of puparium formation in this genotype, which when untreated forms the puparium 7 to 25 days after oviposition (at 25°C). He concluded that the pupation hormone in the lethal-giant larva is produced later or in smaller quantity than in the wild type.

It seems probable that the delay in time of pupation of the heterozygous Minute also is due to an insufficient or delayed secretion of pupation hormone or to the inability of the tissues to react to the hormone. Although no abnormality was found in Weismann's ring in the *Mw* larvae studied histologically, the staining technique was not one with which secretory action of the cells could be detected.

The second effect of the Minute on development, delay in time of occurrence of the larval moults, can less readily be attributed to a known agency. That moulting in holometabolous insects is controlled by a hormone has been demonstrated only for the Lepidoptera. BUDDENBROCK (1930), working with Sphinx and Dilina, injected body fluid from caterpillars which were about to moult into caterpillars which had just moulted, and noted accelerated moulting in the latter. BODENSTEIN (1933), in transplantation experiments on Vanessa, found that bristle and leg transplants of different age than the host moulted at the same time as the host. No study of hormonal control of moulting in the Diptera has been found in the literature.

The fact that development of the heterozygous Minutes is characterized by delay in larval moulting and also by delay in pupation, while the homozygous Minutes do not moult at all, suggests the possibility that in Drosophila the stimuli to moulting and to pupation are one and the same. BUDDENBROCK (1930) is in fact of the opinion that in the Lepidoptera the moulting and pupation hormones are qualitatively the same. He bases his opinion on experiments in which the blood of caterpillars ready to pupate, injected into younger caterpillars, usually induced pupation but occasionally induced moulting. The validity of BUDDENBROCK's methods has been questioned by other workers (PLAGGE 1938), and no relationship between

the two processes has been conclusively established in the holometabolous insects.

As stated in a previous section, two studies of larval growth have revealed an alteration of the rate of growth through the agency of genetic factors, those of vestigial by ALPATOV (1930) and of chubby by DOBZHANSKY and DUNCAN (1933). Whether the Minute case resembles that of chubby in a changed ratio of width to length from the beginning of larval life cannot be decided from the present data. Since most of the larval tissue grows by increase in cell size, it is difficult to picture such cell growth occurring at different rates in two dimensions. It is possible that, at the end of the embryonic period, the Minute and wild type larvae may differ appreciably in the number of cells in the width of the larva, and slightly in the number of cells in the length of the larval body.

Relatively few phenotypes of Drosophila have been studied by examination of the imaginal disks of the larvae. Several were investigated by CHEN (1929), who found that in Bar, lozenge and eyeless zygotes, the optic disks are reduced in size in the larval stage; while in vestigial and no-wing the dorsal mesothoracics are smaller than the wild type, and the dorsal metathoracics are increased in size in larvae homozygous for bithorax. His observations on vestigial have been confirmed by AUERBACH (1936) and those on eyeless have been corroborated by MEDVEDEV (1935). MEDVEDEV found that in Lobe and glass larvae, also, the optic disks are smaller than in the wild type. An effect of genetic factors upon the imaginal disks during the larval period has therefore been demonstrated. In only one of these cases, however, has larval growth been studied. ALPATOV (1930), by means of length measurements, found that larval growth is slower in the vestigial homozygote than in the wild type, and that vestigial larvae never attain the size of the wild type. Here the effects on larval and imaginal tissue are similar; both are retarded in growth rate. The effect of the heterozygous *Mw* factor in altering the growth of both larval and imaginal tissue is therefore not an isolated case.

The *Mw*, *MFla* and *Ml*^{2} homozygotes appear to show the same kind of effects as those exerted upon the heterozygotes, but to a more extreme degree. The process of growth is greatly if not entirely inhibited, while moulting does not occur at all. However, nothing is as yet known about the imaginal tissues of the homozygotes.

Although a close relationship is highly probable between the four characteristics of the development of the Minute heterozygote described above, no known mechanism explains their occurrence as pleiotropic effects of the same genetic factors. It is tentatively suggested that some material exists in the larva which is used by the tissues in growth and also in manufacture of the pupation hormone and of the stimulus to moulting. The heterozy-

gous Minute factor may control the production of this material in such a way that the available quantity of it is less than that in the wild type, with the result that the rate of tissue growth is altered and moulting and pupation are delayed. In the homozygote, this hypothetical material might not be produced at all or in sub-threshold quantity, so that moulting does not occur and the process of growth is interrupted.

Such an hypothesis has an insecure foundation in the present state of knowledge of larval development, and must wait for confirmation until the processes of tissue growth, moulting and pupation have been thoroughly studied from the physiological point of view.

ACKNOWLEDGMENTS

The writer is grateful for his interest and advice to Professor L. C. DUNN, who suggested the problem and under whose supervision it was investigated. She wishes to thank DR. NATHAN KALISS for his advice in matters of histological technique, and MR. JACK GODRICH and DR. DIETRICH BODENSTEIN for their help in making the illustrations.

SUMMARY

A study of flies heterozygous for Minute mutations was made with the object of ascertaining the effects of Minute factors on development. The following facts were established:

1. Although its developmental period is prolonged, the Minute fly (*Mw*, *MFla* and *Ml*^{2}) is significantly smaller than the wild type, taking tibia length as an index of body size.

2. *Mw* and *MFla* larvae form puparia 41 to 43 hours later than their wild type sibs. The pupal period is prolonged 12 to 21 hours.

3. The *Mw*, *MFla* and *Ml*^{2} zygotes are not delayed in hatching from the egg.

4. There is no sex difference in time of hatching from the egg or in length of the larval period of the wild type or Minute zygotes. In both genotypes, the pupal period of the male is longer than that of the female.

5. The *Mw*, *MFla* and *Ml*^{2} larvae pass through the usual three larval instars; the prolonged larval period is not therefore due to an additional instar.

6. There is no great difference between *Mw*, *MFla* and *Ml*^{2} heterozygotes and the wild type in larval length at 24, 36, 48 and 96 hours after hatching from the egg; in the case of *Mw*, the Minutes are slightly shorter at 100 hours (beginning of puparium formation of the wild type) and increase slightly in length during the next 44 hours. At 100 hours the width of *Mw* larvae is significantly less than that of wild type. There is, therefore, an effect of the Minute condition upon growth of the larva.

7. At 96 hours after oviposition and later, both wild type and Minute larvae show sexual dimorphism in larval size; the females are larger.

8. The *Mw* and *MFla* larvae undergo the first larval moult at an average of one hour later than their wild type sibs, and the second moult three hours later than the wild type.

9. Histological study of the larvae shows that certain imaginal disks of the *Mw* larvae (ventral thoracic, dorsal meso- and metathoracic and antennal) are less advanced in size and degree of differentiation at 48 and 72 hours after hatching from the egg (early and late third instar) than are those of the wild type of the same age. The Minute optic disks are smaller than the wild type at 48 hours, but at 72 hours no difference could be discerned. At 120 hours, the imaginal disks of the *Mw* larvae are in an advanced stage of growth and differentiation, and indicate that these processes continue during the time of prolongation of the larval period. The size of Weismann's ring is variable in both *Mw* and wild type larvae at 48 and 72 hours; no constant difference in this gland was observed between the Minute and wild type larvae. No difference in morphology of the larval tissue was observed between the *Mw* and wild type larvae. These observations must be regarded as preliminary, because of the small number of specimens studied.

10. The *Mw*, *Ml*[2] and the majority of the *MFla* homozygotes die during the first larval instar; a small proportion of the *MFla* homozygotes die just before hatching from the egg. The homozygous larvae grow only very slightly and do not moult.

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THE RELATION BETWEEN THE COLOR OF TESTES AND VASA EFFERENTIA IN DROSOPHILA*

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Received October 31, 1938

IN *Drosophila melanogaster*, as in the diptera in general, the genital ducts are independent in origin from the gonads. The ducts are outgrowths of imaginal discs, located at the posterior end of the larval body, which join during the pupal metamorphosis with the gonads which themselves at this stage lie embedded in the fat body in the body cavity. In adult males of the wild race of *D. melanogaster*, as well as in most mutant races, both the testes and the vasa efferentia are yellow or orange colored. On the other hand, in stocks where the eye pigmentation is very light or where no pigment is formed, both testes and vasa are colorless or nearly so.

In order to study the origin of male sterility due to absence of a complete Y chromosome we have transplanted sterile and fertile testes into fertile and sterile larval hosts respectively (STERN and HADORN 1938). For purposes of identification, fertile testes from a nearly colorless race and sterile testes from a colored race were used. During these studies a dependence of the coloration of the vasa derived from the host upon the testis derived from the donor became apparent. This was confirmed in experiments especially devoted to the problem. The following forms an analysis of the testis-vas interrelationship (STERN and HADORN 1937).

METHODS

The transplantations were made with the technique of EPHRUSSI and BEADLE, generally by using larval hosts of the last instar and testes from slightly younger donors but still of the same instar (see STERN and HADORN 1938). The main body of evidence was obtained in *Drosophila melanogaster*. In the original experiment the colored testes were of the constitution "carnation" (*car* at I, 62.5) and the nearly colorless testes "apricot vermilion" (w^a at I, 1.5; *v* at I, 33.0). Later only testes of "wild" constitution were used for "colored" gonads and either the slightly tinged $w^a v$ or white (*w*) testes for colorless ones. It will be seen from tables 1–4 that in regard to the color of the vasa carnation behaves like wild and apricot vermilion like white. This latter result was somewhat unexpected since transplantation experiments (EPHRUSSI and BEADLE 1937) have shown differences between *w* and $w^a v$ with respect to

* A part of the cost of the accompanying illustrations is met by the Galton and Mendel Memorial Fund.

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eye color. The reason for the divergence in regard to vas and eye coloration will become clear in the discussion.

In the first series the operated specimens were dissected about the fifth day of adult life. Later all dissections were made 24–48 hours after emergence from the pupa. No significant differences in coloration were noticed between the earlier and the later dissected cases. In the following account "colorless" and "not-colored" refer to vasa and testes of both *w* and $w^a v$ constitutions.

Our experiments with *D. melanogaster* have been supplemented by results on *D. pseudoobscura*, kindly put at our disposal by DR. TH. DOBZHANSKY and by interspecific transplantations between some other Drosophila species.

COMPOUND TESTES

After a successful operation the implant either develops "free," that is, without becoming attached to a vas, or it replaces a host testis by joining

FIGURE 1.—Internal male genitalia of *Drosophila melanogaster*. Semidiagrammatic after SHEN. *t* = testes; *ve* = vasa efferentia; *pr* = paragonia; *vd* = vas deferens.

with a vas, or finally it fuses with one or rarely both host testes, thus forming compound gonads. These were always found to be connected with a vas. In the stage in which the testes were transplanted the gonads are ellipsoid bodies. A free testis retains its larval oval shape in spite of increase in size while attachment to a vas leads to a transformation of the testis into a long spiral (DOBZHANSKY 1931; STERN 1938). DOBZHANSKY and BEADLE (1936), transplanting testes in *D. pseudoobscura*, found a percentage frequency of the three main types of implant development of 61.1 ± 4.5 free; 27.3 ± 4.2 separately attached; 11.5 ± 3.0 compound. In our work with *D. melanogaster* the first series gave the percentage proportions $66.2 \pm 5.6 : 15.5 \pm 4.3 : 18.3 \pm 4.5$ (data given in STERN and HADORN 1938, table 1) while in the later and larger series (this paper, tables 1 and 3) nearly all implants which were not "free" had formed compound gonads ($58.6 \pm 4.3 : 1.5 \pm 1.1 : 39.9 \pm 4.3$). Whether these diverse results are due to genetic differences in the races employed, or to differences in age of host and donor or to other causes, is unknown.

The diagnosis of a testis as a compound is of importance for the later

analysis and a description of the criteria, therefore, is in order. Except for a few special cases, a gonad was considered to be compound (1) whenever its membrane was a mosaic of colored and uncolored areas and (2) when no free implant was found simultaneously. In addition, in the very great majority of cases such a gonad indicated its compound nature also morphologically by its larger size when compared with the normal testes of the same individual and by its obviously double structure—from a nearly separate round testis attached on the side of its spiral partner, to a uniform spiral gonad with two separate apical endings. That the two parts which make up compound testes actually enclose a common cavity was seen both in some fresh dissections as well as in sections of a few selected cases. This was also apparent from the fact that in compounds of sterile

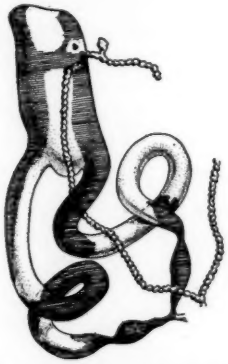

FIGURE 2.—*D. melanogaster*. Triple compound gonad consisting of two colored (+) host testes and a white donor testis. A malpighian tubule is partly enclosed by the compound structure.

and fertile testes motile spermatozoa in the appropriate vas were present even when the fertile part, as judged by its color, was mainly restricted to the apical end. However, it must be pointed out that the distribution of the colored and uncolored areas on the surface of a testis may not necessarily permit deductions as to its internal structure. This will be discussed below.

The exact mode of compound formation is unknown. For its understanding it may become of importance to point out that in four cases out of a total of 50 compounds, a triple structure was formed. Here the two original host testes had become connected with each other by the implant which had fused with each of them. One such case is shown in figure 2. In addition to the general characteristics of a triple compound, this case is distinguished by a special feature. A malpighian tubule obviously has been "caught" during the process of fusion of the gonads so that it pene-

trates the compound structure. The tubule was found to lie free within a tunnel-like duct formed by the walls of the gonad. It seemed probable that the walls of the duct were partly provided by one yellow host testis and partly by the white implant. The formation of this strange structure points to the assumption that the initial fusion of the testes into compounds may occur independently in more than one region of the two joining gonads.

In addition to these triple compounds there were two cases in which the implant had become divided into two parts each of which had gone into compound formation with one host testis. Perhaps these cases are derived from triple compounds. In another instance two separate (but attached) gonads, obviously compound, had been formed by the implant and one host testis while the other host testis had remained free.

IMPLANTATIONS OF WILD TYPE OR *car* TESTES INTO *w* OR $w^a v$ HOSTS

In the great majority of cases *a free implanted testis of wild constitution does not exert any visible influence on the pigmentation of either $w^a v$ or w vasa.* Table 1 indicates this for 45 out of 47 cases, if we consider the first two

TABLE 1

Testis transplantation experiments in D. melanogaster (colored testis into colorless host).

TYPE OF TRANSPLANTATION	TOTAL	I		II	III			IV
		IMPLANT FREE		IMPLANT ON TIP OF ONE HOST TESTIS; PROBABLY NO COMPOUND	ONE COMPOUND ATTACHED			BOTH TESTES COMPOUND OR TRIPLE COMPOUND
		VASA COLORLESS	ONE VAS WITH COLORED SPOTS	VASA COLORLESS	BOTH VASA COLORLESS	1 VAS COLORLESS; 1 VAS COLORED	BOTH VASA COLORED	BOTH VASA COLORED
$+ \rightarrow w^a v$	25	17*	—	—	1	2‡	4	1
$+ \rightarrow w$	42	17	2†	11	—	1¶	10	1

* In one individual one host testis and its vas were degenerate.
† One case: one spot; one case: three spots.
‡ One case doubtful if really compound testis.
¶ One case doubtful if really compound testis (figure 2).

classes together. In reality the first group only carries the designation "Implant free," while the second group is labelled "Implant on tip of one host testis, probably no compound." This distinction had not been drawn

carefully in the series $+ \rightarrow w^a v$, so that it is likely that the 17 cases of the column "implant free" in reality contain some specimens which belong to the next column. The importance of distinguishing the two groups comes from indications of a closer relation of host and implant gonad in these cases of rather loose attachment to the tip of the former. In nine of the 11 individuals yellow pigmentation like that of the attached implant was found to cover narrow adjacent parts of the otherwise colorless host gonad and at the same time small colorless areas or islands were found on the otherwise colored implant. A case of this nature, as far as the gonads were concerned and disregarding the vas, is shown in figure 3. In the remaining two of the 11 individuals no yellow pigment was found on the host gonad, but an unpigmented region occurred on the implant. As stated in table 1 none of the 11 implants and host testes which were attached to each other

FIGURE 3.—*D. melanogaster*. Host: white; donor: +. Implant externally attached to host gonad. Colored area on host and uncolored area on donor testis. One vas partly colored.

had formed a real compound gonad. The criterion on which this judgment is based was the lack of a communication between the two gonadal cavities. It is, however, possible that the mosaic pigmentation in the cases of the two gonads indicates a limited compound formation between their membranes. It must be added that in two cases the host testis of the connected pair possessed a large yellow island somewhere along the middle portion of its otherwise colorless spiral. A discussion of these interrelations of the pigmentation of the testes will be given later.

There were two exceptions to the rule that the vasa remain uncolored in the presence of a free colored implant. In these two individuals one of the two vasa still fitted the rule by having remained unpigmented; the other vas, however, possessed in one case one, in the other case three yellow spots. The single spot was located near the middle of the vas, on one side of it. Of the three spots two were situated at that end of the vas into which the gonad opened and the third at the opposite end. The pigmented areas in the two exceptional vasa covered considerably less than 25 percent of the total surface of each vas.

When we consider the ducts to which true compound gonads had become attached, we find more numerous and more striking examples of *yellow*

pigmentation on vasa of colorless constitution. In only one of 18 cases which have been classified in the third group of table 1 was there no trace of pigment to be found on either vas. In three of the 17 positive cases the pigment was seen only on that vas efferens which connected the compound gonad with the vas deferens and in the remaining 14 cases both vasa efferentia were colored. To these last cases may be added two more, recorded in the next (fourth) column of table 1.

An analysis of the types of compound testis discloses that a correlation exists between presence of pigmented testicular areas near the vas and the amount of coloration on the vas itself. In the individual both of whose vasa were colorless the compound gonad consisted of a long spiral colorless part and a smaller club-shaped colored part joined to the former in its apical half only. No pigment was present on any region of the lower half of the

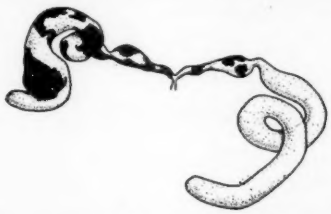

FIGURE 4.—*D. melanogaster.* Host: white; donor: +. Compound gonad. Both vasa spotted, the left to a higher degree than the right.

compound gonad. There is a slight possibility that no true compound gonad had been formed in this case. This possibility is greater in two of the three cases where one vas had remained completely colorless while the other exhibited pigmentation. In one case the implant was located at the tip of a host testis which itself was completely colorless, one small spot excepted. Only the uppermost, in this case unusually constricted portion of the vas appeared clearly orange colored. The second doubtful specimen exhibited the condition shown in figure 2. Again a very large lower section of the possibly compound testis was unpigmented. This time a considerable area of the vas *distal* to the connection with the testis was pigmented. In the third case we meet for the first time a completely yellow vas. It was attached to a compound testis the lowest region of which was yellow and which alternated with more apical colorless and yellow regions.

When we come to the 14 specimens in which pigment was found in both vasa though a compound gonad was attached to only one of them, we find in 10 cases that the amount of pigmentation was greater in that vas which belonged to the compound testis than in the other vas (figure 4). In the four other cases no striking differences in total size of pigmented area were found; in one of these individuals both vasa were completely

colored. In no case was the vas, which was connected with the uncolored host testis more colored than the compound attached vas. Among the 10 pairs with differences in amount of color one case was found with the first vas about 50 percent colored, and the second vas about 10 percent colored; one case with the first vas about 80 percent colored, and the second vas less than 30 percent colored; and eight cases with one vas fully colored (in two cases perhaps only 95 percent), and the other vas from about 10 to about 50 percent colored (in one case about 80 percent).

Finally all vasa of each of the two individuals with double or triple compound gonads were colored in from 25 to 90 percent of their area.

These findings concerning wild-type implants are supplemented by six specimens from the series *car*→$w^a v$ (table 2) where the implanted testis

TABLE 2

Testis transplantation in D. melanogaster.

TYPE OF TRANSPLANTATION	IMPLANT ATTACHED WITHOUT COMPOUND FORMATION	
	VAS ON IMPLANT COLORED	BOTH VASA COLORED
car→$w^a v$	4*	2

* In one case the two host testes were free, and only one vas was present to which the implant had become attached.

had become attached to a vas separately, that is, without fusion with a host testis. In each case the vas belonging to the colored implanted gonad was fully pigmented (figure 5). Moreover, in two individuals the coloration was also partly extended over the other vas.

FIGURE 5.—*D. melanogaster*. Host: $w^a v$; donor: *car*. Donor gonad attached to colored vas.

In summing up, the most significant result of the transplantations of testes of "colored constitution" into hosts of "uncolored constitution" is that a ***genetically colorless vas efferens acquires pigmentation when the colored testis has become attached to it*** either by itself or in the form of a compound gonad. In many cases the coloration extends over both vasa though to a different extent. A few cases were found also in which colored spots occurred on a vas of an individual where the implanted testes had not become attached at all.

The location of colored areas on the vasa

There does not seem to be any definite preference in regard to location of the pigmented areas. The colored regions were found scattered at random over the whole surface of the ducts both on the duct which was in direct connection with the implanted testis and on the other duct (figures 3, 4, 6). This should be pointed out especially, as certain selected cases might give the impression of a diffusion-like spreading of the coloring effect. Thus a single colored spot occurs occasionally next to the entrance place of the colored compound testis, or a single colored area may be seen on the lower end of a vas adjoining the totally pigmented vas of the other side. However, other cases where the colored spots are as far away as possible from the larger colored areas show this impression to be erroneous.

FIGURE 6.—*D. melanogaster*. Host: white; donor: +. Vasa efferentia. Attached to the left was a compound gonad the lowermost part of which was uncolored as indicated in the figure. To the right a white testis was attached. Note different total size of colored areas on the two ducts. The fine lines indicate tracheoles. A tracheole forms the border of the unpigmented area farthest to the left.

In this connection it may be mentioned that in those individuals in which the two vasa efferentia open into the vas deferens by means of a short common stalk containing the two ducts this stalk and the immediately adjacent parts of the vasa proper are frequently unpigmented even when the whole remainder of one vas, that is, nearly the complete vas and a large lower section of the other vas are colored.

The outline of spots both on the compound testes and on the vasa was quite definite and without intergrading. Frequently the limiting borders formed sharp angles. There were no outlines which pointed to a simple diffusion of color. In some cases it was seen that a limit of an area coincided exactly with the course of a tracheole in the testicular sheath; however, in many other cases no such relation existed (figure 6).

IMPLANTATIONS OF *w*^{a}*v* AND *w* TESTES INTO WILD-TYPE AND *car* HOSTS

In all 31 specimens with a free implant both vasa were completely pigmented (table 3, I). The implanted testis generally remained devoid of color and the host testes were fully colored. However, in a number of cases in which the implant had become connected with the tip of a host testis

small colored areas on the implanted testis were visible near the border between the two gonads.

The majority of specimens with one compound testis (22 out of 28 in table 3, II) also possessed two completely colored vasa (see figure 1a in STERN and HADORN 1938). In nearly all of these cases the lowermost region of the compound gonad possessed pigmented areas. In three of the six specimens with colorless areas on that vas, into which the compound gonad opened, an extensive unpigmented region formed the lower end of the testes.

TABLE 3

Testis transplantation experiments in D. melanogaster (colorless testis into colored host).

TYPE OF TRANSPLANTATION	TOTAL	I IMPLANT FREE OR ON TIP OF ONE HOST TESTIS	II ONE COMPOUND ATTACHED		III TWO COMPOUNDS ATTACHED	IV TRIPLE COMPOUND		V IMPLANT ATTACHED WITHOUT COMPOUND FORMATION	
		BOTH VASA COLORED	1 VAS SPOTTED	BOTH VASA COLORED	BOTH VASA COLORED	1 VAS SPOTTED	BOTH VASA COLORED	1 VAS SPOTTED	1 VAS COLORLESS
$w^a v \rightarrow +$	15	8	1*	5	1†	—	—	—	—
$w \rightarrow +$	48	23	5‡	17¶	1	1**	2	1††	1

* Colorless areas about 50% of total vas.

† Colored testis free.

‡ Three cases: one small colorless spot in middle of vas; two cases: colorless area about 50% of total vas.

¶ One case: compound-nature doubtful.

** Only small colorless spots on yellow background

†† Only small colorless spot on yellow background.

There were five individuals with either two compound testes each, or one triple compound (table 3, III and IV). In four of them both vasa were completely pigmented and in the fifth only small spots had remained colorless. In all five cases the testes possessed yellow areas near or directly adjacent to the vasa.

Of greatest interest in these series are the seven individuals in which the colorless implants had become attached to a vas without having entered into a compound formation (table 3, V, and table 4). In two cases this vas was largely colorless and in five it was completely so. In three of the latter cases the other vas had not become pigmented, once apparently due to generally poor development and twice in connection with the fact that no gonad had become attached to it at all, leaving both host testes free. The two other cases, however, showed a complete development of the uncolored vas.

The general conclusion from the results of implantations of colorless gonads into colored hosts is that *the vasa efferentia of the genetic constitution "colored" are usually not able to exhibit pigmentation when connected with an unpigmented gonad.*

TABLE 4

Testis transplantation in D. melanogaster.

TYPE OF TRANSPLANTATION	IMPLANT ATTACHED WITHOUT COMPOUND FORMATION	
	ONE VAS SPOTTED	ONE VAS COLORLESS
w^{a}v→car	1*	3†
w →car	—	1‡

* One colored spot of less than 30% of total area.

† One case: other vas colored.
Two cases: no testes attached to other vas, *colorless!*

‡ Other vas very underdeveloped. Attached to a yellow host testis but itself colorless.

TRANSPLANTATION IN DROSOPHILA PSEUDOOBSCURA

Extensive unpublished data relating to *Drosophila pseudoobscura* were obtained by DR. TH. DOBZHANSKY and with his permission are presented here. Three different stocks of this species were used: (1) the wild type which is characterized by deep orange-red coloration on testes and ducts, (2) the prune mutant exhibiting a pale yellow testis-duct color, and (3) an eosin magenta strain with colorless testes and ducts. The two last named stocks behaved so similarly in transplantation experiments that they have been combined in tables 5 and 6 which were compiled from DOBZHANSKY'S original notes. It seems unnecessary to discuss the results in detail. A study of the tables makes it obvious that a dependence of the coloration of the vasa efferentia upon that of the attached testes exists, very similar to that in *D. melanogaster*. Special attention should be paid to the recorded distribution of pigment in compound testes or in testes which are externally attached to each other. Irregular splashes or spots of color are frequently found on genetically unpigmented gonads.

INTERSPECIFIC TRANSPLANTATION

Interspecific transplantation, made reciprocally between the eosin magenta stock of *D. pseudoobscura* and normal *D. azteca*, and reciprocally between the same *D. pseudoobscura* stock and *D. virilis*, again demonstrate the relation between testis and vas color. The testes and vasa of *D. azteca* and *D. virilis* are reddish-orange. In more than 20 cases spots of this color were found on testes and vasa of genetically unpigmented constitution where attachment of donor gonad to host duct, compound formation or close external junction of host and donor testes had occurred. It is note-

worthy that in these cases the interrelation of tissues is based upon transplants derived from a species different from the host species.

TABLE 5

Transplantation experiments in D. pseudoobscura. Donor: wild; host: prune or eosin magenta. Color of implant: red.

RESULT OF EXPERIMENT	HOST		NO. OF CASES
	TESTES	VASA	
free or only touching a host testis	uncolored	uncolored	27
compound	part of compound red	one or both fully or spotted red	8
attached	uncolored	both fully or spotted red	9
attached	part of loosely touching host testis spotted red	both fully or spotted red	5
attached (small)	part of loosely touching host testis spotted red	adjoining vas red, the other uncolored	1
attached to both vasa	uncolored	red	1
not found	uncolored	1 red spot	1

TABLE 6

Transplantation experiments in D. pseudoobscura. Donor: prune or eosin magenta; host: wild. In all cases testes and ducts of host: red. Implant slightly tinged or uncolored unless stated otherwise.

IMPLANT		NO. OF CASES
free	uncolored	13
loosely touching a host testis	red spot near contact	5
compound	nearly fully red	2
attached	uncolored	1
attached	red spots near contact zone with host testis and near vas	1

THE DEVELOPMENTAL RELATION BETWEEN TESTES AND DUCTS

If it is true that the testes and ducts originate completely independently from each other, then the observed interaction has to be interpreted as a

non-autonomous behavior of the pigment-producing cells of host and donor. However, such an interpretation meets difficulties. It involves the assumption of diffusion of a specific substance from an attached colored testis to account for the pigmentation of the vasa. Such a diffusion process would have to depend on more or less direct contact of a vas with a pigmented region of an attached testis, for the vasa generally do not acquire color when the colored implant is free or when the lower parts of the attached testes are colorless. Special assumptions are necessary in order to explain the facts pointed out earlier that the outlines of colored areas are clear-cut without intergradations of color and that they often form sharp angles and straight lines. Further difficulties arise in adapting a simple diffusion hypothesis to certain specific mosaic color conditions. If the source of diffusion is postulated to be at the junction of testis and duct it is hard to see why occasionally only the opposite end of the vas is colored or why sometimes only distant parts of the other vas are pigmented when the nearer regions are transparent.

It might appear possible to overcome the objections raised by the addition of further assumptions to the diffusion hypothesis. It seemed more appropriate, however, to open once more the question of the ontogeny of testes and vasa and their supposed independence. Clearly most difficulties would disappear and the results would become fully intelligible if the origin of the vasa could be traced back to the gonads.

The independent origin of the genital ducts of flies and of the gonads was first demonstrated by BRÜEL (1897). In *Drosophila melanogaster* these observations were corroborated by DOBZHANSKY (1930) who made a complete series of pupal dissections showing the gradual transformation of the genital imaginal disc into the various ducts and accessory parts of the internal genitalia and their gradual joining up with the gonads which do not appear to contribute to the ducts. On the other hand, at nearly the same time, GEIGY (1931) expressed the view that contrary to BRÜEL's observations, the vasa efferentia are derived from a special group of cells in the testes and that only the vas deferens owes its origin to the imaginal disc.

Although GEIGY has failed to document his statements by means of illustrations, while DOBZHANSKY has produced a clear series of drawings, it seemed desirable to reinvestigate the problem once more. MR. H. D. STALKER, in our laboratory, prepared and studied paraffin-sections of pupae of consecutive stages between 0 and 52 hours after pupation, kept at about 22.5°C. His conclusions agree in the main with those of DOBZHANSKY reached by total dissections. It can be seen without doubt that the imaginal disc produces, among other structures, two hollow tubes which grow forward till they meet the testes and transform into the vasa efferentia.

These observations of the normal development are further substantiated by observations of occasional abnormalities. It happens sometimes in gynandromorphs that normal vasa are present in spite of the absence of testes (*D. simulans*, DOBZHANSKY 1931). Similarly in transplantation experiments cases occur in which both vasa are present although only one testis is found attached to a vas while the other testis or testes are found free or are lacking. On the other hand, in transplantation work no indi-

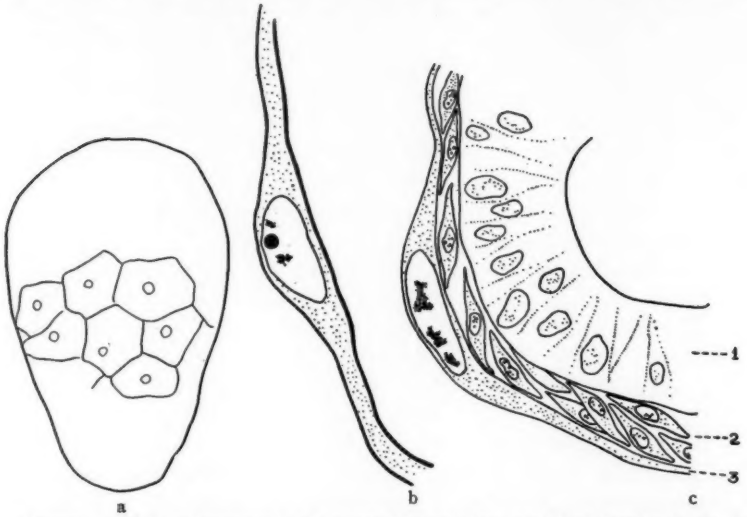

FIGURE 7.—(a) *D. melanogaster*. Adult, unattached testis from stock "sx" showing clearly the single layer of epithelial cells. Only some of the cells are indicated. Drawn from living gonad. 360×.

(b) *D. pseudoobscura*. Cross section through a sheath cell of adult testis. Permanent preparation. 1560×.

(c) *D. melanogaster*. Cross section through vas efferens of 40 hour pupa about 10 hours after attachment of testis to vas. Permanent preparation. 1560×.

(1) inner epithelium of vas
(2) outer epithelium of vas
(3) sheath cell.

vidual has ever been found with more than two vasa though three or even four testes were present in the adult, either partly free or as compound gonads.

While thus a study of the gonads and ducts as whole organs seemed to demonstrate their independence, a closer histological investigation finally has established that certain cells of the vasa efferentia are derived from the testes. An examination of the testes of adult wild males showed that the yellow color is caused by a single layer of pigment granules which are located in the external epithelial covering of the gonads. This covering

consists of very characteristic, large, flat cells, the nuclei of which bulge out over the otherwise low cytoplasmic epithelial level (figure 7a, b). It was found that the same type of cell covers the unpigmented testes but that these cells lack pigment. Furthermore, it became obvious that the external cover of the vasa efferentia consists of precisely the same type of cells as that of the testes and that no such cells are found in other parts of the genital tract.

FIGURE 8.—Host: *D. pseudoobscura* (eosin magenta). Donor: *D. virilis*.
(a) Donor gonad externally attached to host gonad. Two colored areas on host testis, one consisting of single cell.
(b) This cell more highly magnified.
(c) Single cell forming a colored spot in a similar case. To the right a tracheole.

The ontogenetic history of these cells during the pupal stages was studied in MR. STALKER'S slides. At the time of pupation the typical

though colorless epithelial coverings of the testes are present while no similar cells occur in the imaginal disc of the ducts. This condition persists through the following stages. No striking changes occur in regard to the testes. The vasa efferentia, however, become differentiated as outgrowths of the imaginal disc. At this time they consist of small cells which form a columnar layer on the inside and a squamous layer outside. At the time of close approach between the outgrowing ducts and the testes, stages are found which indicate clearly a migration of the large cells of the external testicular covering toward the vasa which in a slightly later

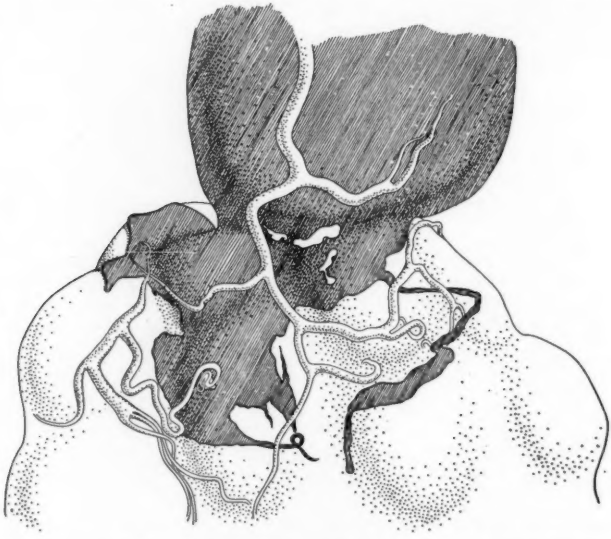

FIGURE 9.—Host: *D. pseudoobscura* female. Donor: *D. azteca* male. Colored testis (above) attached to uncolored ovary (below). Only parts of the gonads are indicated. Pseudopodial overgrowth of pigment from testis to ovary. The light tubes represent tracheae.

stage are covered completely down to the region of junction of the two ducts (figure 7 c).

The observations regarding the derivation of the pigment cells on the vasa efferentia by migration from the pigment cells of the testes provide a simple basis for the interpretation of our transplantation experiments. Accordingly, no question of genetic cell autonomy or depedence in regard to coloration is involved but an ontogenetic derivation of the external epithelium of the ducts from that of the testes.

Further light is thrown on the migration of epithelial cells derived from the testes by observations on adult individuals in the experimental class

"transplantation of *D. virilis* or *D. azteca* testis into *D. pseudoobscura* hosts." In these species the sheath cells are very clearly visible in freshly dissected living organs. It was found that isolated spots of pigment on genetically uncolored host testes sometimes represent single epithelial cells. These cells have an irregular, sharply defined outline suggesting pseudopodial motion (figures 8a, b, c). A still more striking picture was presented in a case of implantation of a *D. azteca* testis into a *D. pseudoobscura* female. The orange-red male gonad had become connected to the tip of one of the unpigmented ovaries (figure 9). From the region of attachment a strikingly pigmented sheet of cells had "flowed" over to the ovary which covered the surfaces of egg-strings and sent out a number of peculiar, long pseudopodial processes.

It would be interesting to study the histology of vasa neither of which has joined a testis in order to check whether the large celled external layer is absent. No such material is available at present.

DISCUSSION

Migration of pigment-producing epithelial cells enables us to understand the general dependence of the duct coloration upon that of the testes, the often irregular distribution of spots, the extension of coloration to both vasa in case of attachment of a colored testis to one of them only, as well as the frequently sharp and irregular outlines of spots. It also explains the few cases of pigmented spots on unpigmented host testes in the presence of a completely free implanted testis as due to migration of some pigment cells from one testis to another possibly during an early pupal stage in which a direct contact may have occurred.

Finally it makes intelligible the occurrence of "tongues" of unpigmented areas reaching from a colorless testis into the pigmented surface of an attached colored testis and also the rare occurrence of unpigmented islands within a pigmented testis cover. Obviously not only the pigmented cells but also their unpigmented homologues are able to move.

This latter point calls for further consideration. In the cases of two vasa to one of which is attached a pigmented gonad to the other a non-pigmented one, it is found that on the average more than 50 percent of the total area of the two vasa is colored. As a general rule it may be stated that in transplantation cases the pigmented cells cover a relatively larger area of either vasa or testes than the corresponding unpigmented cells. Two explanations of this fact may be suggested. It is possible that the initial number, the growth rate and the migratory activity of both cell types is equal and that some of these cells come to lie on top of each other in more than a single layer. Under these conditions a multilayer of pigmented and unpigmented cells might appear as a normally colored

area and not reveal the presence of the colorless epithelial cells. This hypothesis, however, does not seem to be corroborated by observation. Though perhaps not fully conclusive, inspection seems to disprove the presence of more than a single layer of external cells covering the testes and ducts. In this case we should find different grades of coloration, since two or more layers of yellow cells would be darker than one single layer. This is contrary to observation. Furthermore, in favorable instances the cell boundaries can be seen and no overlapping of cells is found. An alternative hypothesis may ascribe different degrees of activity to cells of different genetic constitution. This means with regard to our *D. melanogaster* experiments that wild type cells either have a higher growth or a higher migration rate than genetically white cells. If the growth rate is equal for both kinds of cells, differential migration rates may still lead to different sizes of colored areas provided that the area taken up by single cells varies with the amount of spreading of the cytoplasm.

The general interpretation of the relation between colored and colorless areas seems consistent with the facts described in this paper. However, it does not fit certain facts described by DOBZHANSKY (1931) in *D. simulans* gyandromorphs which possessed a "white" constitution in their male parts and were "wild" in their female parts. These facts are: (1) gradual pigmentation with age of genetically "white" testes in partly "wild" gynandromorphs, (2) colored spots on testes which became attached to oviducts, the pigment being restricted to the surroundings of the point of junction, (3) pigmentation of vasa efferentia in the absence of any testes.

Whether these results are due to the fact that the male organs were present in wild type female tissue from the very beginning of development instead of from late larval stages or whether wild type XX cells can differentiate into pigmented epithelial cells can not be decided at present.

SUMMARY

1. Reciprocal transplantations of larval testes between races with pigmented and unpigmented adult gonads show that the color of the vasa efferentia is dependent on that of the testes attached to them and not on the genetic constitution of the ducts themselves.

2. The specific character of this dependence, namely the frequent occurrence of irregular, sharply outlined spots of pigment, makes an explanation based on the assumption of diffusible substance unlikely.

3. A histological study of the pupal development of testes and vasa efferentia provides the basis for the understanding of the experimental facts: Although otherwise of independent origin, the vasa efferentia obtain their external epithelial covering by a migration of the external covering cells from the testes.

4. The experiments were performed mainly on *Drosophila melanogaster*. Corresponding results appeared in transplantations involving *D. pseudoobscura* (experiments of DR. TH. DOBZHANSKY), *D. virilis* and *D. azteca*.

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THE SEX-DETERMINING MECHANISM OF DROSOPHILA MIRANDA

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Received November 10, 1938

INTRODUCTION

A SERIES of recent papers (DOBZHANSKY 1935; DOBZHANSKY and TAN 1936; DOBZHANSKY 1937), has described the unusual and interesting features which characterize the breeding behavior of *Drosophila miranda* and especially its method of sex determination. These papers have also reported the results of hybridization of *miranda* with the closely related and better known species, *Drosophila pseudoobscura*, and have given a detailed description, based on the study of the salivary gland chromosomes of the hybrids, of the similarities and differences in gene arrangement in these two species. In spite of these studies, there remained much that was obscure about the operation of the sex-chromosome mechanism, and particularly about the manner in which such an organization of the sex-determining material could have been derived from the more familiar sort found in *D. pseudoobscura* and, indeed, throughout the genus Drosophila.

Because of the need for more information on these interesting points, PROF. DOBZHANSKY suggested that I attempt to review and extend his observations. For this, for his kindness in furnishing me with stocks used in the investigation, and for much helpful criticism, I wish to express my gratitude to PROF. DOBZHANSKY. Helpful suggestions for the revision of the manuscript have been kindly given by PROF. F. R. IMMER and PROF. C. R. BURNHAM. To PROF. A. H. STURTEVANT, under whose direction this work was performed, I am deeply indebted for advice and encouragement.

The studies here reported have been sufficient to render more understandable the operation of the sex-determining mechanism of *Drosophila miranda*, and to indicate the manner in which it has been derived from the type present in the common ancestor of *pseudoobscura* and *miranda*.

MATERIAL

The wild-type *D. miranda* strain used most in these experiments was that designated as Olympic 1 (DOBZHANSKY 1935). By means of X-ray treatment a number of mutants have been obtained from this wild-type strain by DR. F. N. DUNCAN, working in this laboratory. Four of these mutants, crossveinless (*cv*), Truncate (*Trn*), plexus (*px*), and Smoky 11 (*Sm 11*) have been very useful in the present investigation. I wish to

thank DR. DUNCAN for kindly permitting me to use them. In one series of experiments use has been made of a strain (Wh 60) collected near Mt. Whitney by PROF. DOBZHANSKY. Another strain (Quilcene 1) has been but little used; it appears to be identical with Olympic 1.

The Texas wild-type stock of *D. pseudoobscura* has been used, and also a number of mutant strains.

REVIEW

Ever since the discovery of the fact that the chromosomes are the material basis of heredity, many investigators have been attracted to the comparative study of the chromosomes of related species, since such studies should yield information not merely about the evolution of a character, but indeed about the evolution of the machinery by which hereditary characters are determined. The genus Drosophila was early recognized as one between whose different species there was considerable variation in chromosome number and morphology. Thus METZ (1914, 1916) described 11 different metaphase configurations found in the 26 species the oogonial chromosomes of which he examined. But the work of METZ showed that in spite of this variation certain regularities were preserved. The haploid groups of 21 of the 26 species agreed in having one tiny dot-like chromosome and five arms, these arms being either separate or joined to form one or two V's. In *D. virilis*, for example, there are five independent rods and a dot, in *D. pseudoobscura* three independent rods, one V and a dot, and in *D. melanogaster* one independent rod, two V's and one dot.

This regularity seemed less surprising at the time it was discovered than it did some years later when the rather common occurrence of reciprocal translocations in natural populations of plants had been shown by many workers, especially by BELLING and BLAKESLEE and their collaborators (BELLING 1927, BLAKESLEE 1929). If reciprocal translocations had become established as frequently in the phylogeny of the genus Drosophila as in Datura it would be hard to understand, for example, why a dot-like chromosome should be found in so many species; why it had not lost its identity through the translocation to it of material from other chromosomes. This difficulty had not long been recognized before its explanation became apparent (STURTEVANT and BEADLE 1936). In natural populations of Drosophila there is a strong selection against translocations, this selection being due to the reduction of fertility associated with translocations in the heterozygous condition. (This would be of much less importance in a plant which, like Datura, is predominantly self-fertilizing.) The same argument applies to inversions which include the spindle attachment region. But inversions which are entirely within one

arm have no appreciable effect on fertility in Drosophila, and are correspondingly common in natural populations.

Thus as two populations of the same species of Drosophila become progressively differentiated from each other until they deserve separate specific rank, one might expect that they would quite commonly come to differ by rearrangements within a single arm, only rarely by rearrangements involving a transfer of genetic material from one arm to another. Two methods are available for studying gene arrangement in related species to see whether this relation holds. The cytological method can be applied only to pairs of species which cross, and involves a minute study of chromosome pairing in the hybrid. It appears that chromosomes like those of the salivary glands of the Diptera must be available if complex rearrangements are to be analyzed by this method. The genetic method involves obtaining a large number of mutant genes in each species and determining their linkage relations. This method may be applied to pairs of species which do not cross, as well as to those which do, though in the former case allelism can only be inferred from appearance.

Of particular interest in the present connection is the application which has been made of this method (CREW and LAMY 1935; DONALD 1936; STURTEVANT and TAN 1937) to two not very closely related species, *Drosophila melanogaster* and *D. pseudoobscura*. As more mutants have become available it has grown increasingly clear that during the divergence of *pseudoobscura* and *melanogaster* each arm has maintained its separate identity, little if any genic material being transferred from one arm to another. In tabular form the correspondence may be represented thus:

pseudoobscura		*melanogaster*
arm	=	arm
XL	=	X
XR	=	III L
II	=	III R
III	=	II R
IV	=	II L
V	=	IV

One striking fact emerges from this comparison. The material which is III L in *melanogaster*, containing the loci of sepia, scarlet, javelin, veinlet, and others, is not autosomal in *pseudoobscura*, but is sex-linked, so that the genes in it are inherited like white or yellow (not like bobbed) of *melanogaster*. This means that while the *melanogaster* male is haploid for one arm, the *pseudoobscura* male is haploid for two arms. It seems clear that *pseudoobscura* is descended from a form in which the male, as in *melanogaster*, carried the III L material in duplicate. The question which demands an answer is: How could this change have taken place? It seems

impossible that it could have taken place all at once, for so large a deficiency would surely make the male inviable. This paper will suggest a plausible mechanism by which it might have taken place gradually.

CYTOLOGY OF MIRANDA AND PSEUDOOBSCURA

It will be well first to review briefly the findings of DOBZHANSKY (1935, 1937) and DOBZHANSKY and TAN (1936) on the cytology of *miranda* as compared with that of *pseudoobscura*. The metaphase chromosome groups of females of the two species are indistinguishable, consisting in each of a pair of dots, three pairs of rods, and a pair of V's. The corresponding salivary gland nuclei contain, as expected, one very short strand and five long ones, striking differences between the two species being here apparent. These differences are best studied in the salivary glands of the female hybrids, where each nucleus contains a complete haploid set from each species.

The results of such a study were reported by DOBZHANSKY and TAN (1937). They found that the two species differed by a great many rearrangements involving a minimum of 49 points of breakage. The great majority of the rearrangements involved a transfer of material from one point to another in the same arm. There were, however, six cases which could be interpreted as inter-arm transfers. Since *pseudoobscura* is morphologically so similar to *miranda*, and so different from *melanogaster*, it cannot but seem strange that the first pair should differ by even as many as six translocations while the second pair seem to differ by few if any. DOBZHANSKY and TAN emphasized that some sections were only very rarely seen paired; some, indeed, only a single time. Needless to say, this adds to the difficulty of an already difficult observation. It is particularly unfortunate that critical figures are so rare, for from a study of DOBZHANSKY and TAN'S figure 6, it does not appear that the translocation interpretation is the only one possible. (The difficulties and uncertainities attendant on these observations may be inferred from the case of the dot (V). DOBZHANSKY and TAN had concluded that the dots in the two species were totally different. DOBZHANSKY (unpublished) has made further observations which show that they are the same in every visible detail, the earlier interpretation having been a matter of incorrect identification.)

But whether or not translocations exist, they are certainly neither numerous nor large, from which it follows that each arm of *pseudoobscura* is at least approximately homologous to one of *miranda*.

In this paper the designations XL, XR, II, III, IV and V will be applied to the chromosomes of both species. Following DOBZHANSKY, III of *miranda* will also be referred to as X^2. This is done because the chromosome of *miranda* which is approximately homologous to III of *pseudoobscura* is

not an autosome, but a sex chromosome. This fact does not appear from a study of the females and the female hybrids, but is shown by a comparison of the males.

In mitotic metaphases of the *pseudoobscura* male there are three pairs of rodlike elements, a pair of dots, a V-shaped X, and a Y of which the shape varies in different stocks. In the salivary gland nucleus we find three long strands corresponding to the three pairs of autosomes, two strands, also long, but paler and more slender, corresponding to the unpaired right and left limbs of the X, and one very short element, the paired dots (V). As in all species of of Drosophila heretofore described, the Y is an inconspicuous element entering into the composition of the chromocenter.

In the male of *miranda* DOBZHANSKY found the mitotic chromosomes to consist of a V (X^1), a J-shaped element (Y), a pair of dots (V), 2 pairs of rods, and one unpaired rod. The presence of an unpaired rod was totally unexpected, but an examination of the salivary gland chromosomes showed that one of the strands which in the *pseudoobscura* male was plump and darkly staining, namely III, was pale and slender in *miranda*, like XL and XR, confirming its unpaired condition. Since the approximate homologue of III was paired in the female and unpaired in the male, DOBZHANSKY referred to it as X^2. These observations seemed to point to the extraordinary fact that the *miranda* male was deficient for the entire III, which in *pseudoobscura* would certainly be lethal. DOBZHANSKY and TAN (1936) find it necessary to conclude that "*miranda* and *pseudoobscura* represent two very different reaction systems." (While the paleness and slenderness of X^2 in the male are not entirely dependable as a basis for concluding that it is haploid rather than diploid, the conclusion that it is haploid is strongly supported by observations to be presented below.)

To recapitulate, there are three strands (XL, XR, and III) which are present twice in the female *miranda* and once in the male, two of the three being joined to form a V. (From the evidence thus far presented it would not be possible to conclude, as I have tacitly done, that the two arms which compose the V are, as in *pseudoobscura*, XL and XR. Evidence to be presented below (cf. Exceptional Hybrid Males) excludes the alternative possibilities that III and XL or III and XR might form the V, XR or XL respectively being the rod.) The J-shaped chromosome is present in the male but not in the female, and may therefore be called the Y.

How would such a sex chromosome mechanism work? It is evident that in order for it to perpetuate itself without the formation of inviable gametes or zygotes a non-random segregation of X^1, Y, and X^2 in the meiotic divisions of the male would be necessary. Y would have to go to one pole and X^1 and X^2 to the other. From a cytological study of meiosis (DOBZHAN-

SKY 1935) it appeared that X^1 and Y conjugated and disjoined in a regular fashion, whereas X^2 went to one or the other pole without having conjugated with either X or Y at all. It was unfortunately not possible to say whether X^2 always went to the same pole as X^1, for X^1 and Y could not be distinguished at this stage. But since there was no evidence for the formation of non-functional sperm, and the zygote mortality was too low to account for more than a very small frequency of non-regular disjunction, it appeared that determinate segregation must be the rule (DOBZHANSKY 1935). The further study of this unusual phenomenon was a primary object of the present investigation, the results of which follow.

ACTIVE MATERIAL IN THE Y

An examination of the salivary gland chromosomes of the *miranda* male (MACKNIGHT 1938) showed that in addition to the complement of chromosomes described by DOBZHANSKY and TAN (1936) there was in each nucleus an element composed of many short, banded sections, most of them merging into heterochromatic material at both ends (fig. 1). No such element is present in females. It follows that the element in question is derived in ontogeny from the J-shaped mitotic chromosome which, by virtue of its presence in males and not in females, has already been defined as the Y.

This unexpected finding seemed from the first to resolve many of the puzzling problems connected with the operation and evolution of the sex-determining mechanism of *miranda*. In the first place, it was no longer necessary to suppose that the male was haploid for the entire third chromosome; the inference seemed justified that the *miranda* male carried most if not all the material of III in duplicate, once in X^2 and once in the Y. Such an assumption leads one to ask whether the total length of the euchromatic sections in Y is equal to that of X^2. For several reasons it is difficult to give an exact answer. The absolute and relative lengths of chromosomes vary from nucleus to nucleus depending on preexisting differences and on the amount of artificial stretching. In the present case there is also a source of systematic error, since a short section attached at both ends would not be expected to stretch in response to pressure in the same way as a long strand attached at one end. With these reservations it may be stated that the total length of euchromatin in the Y is little if at all less than that in X^2, both being about 110μ in large nuclei. This observation evidently supports the hypothesis.

In the second place, if we take the Y to be homologous not to X^1 alone, but part to X^1 and part to X^2, then we might expect that trivalent association would occur. Were this the case, the directed segregation of the sex-chromosomes, while still unexplained, would find a close parallel in the

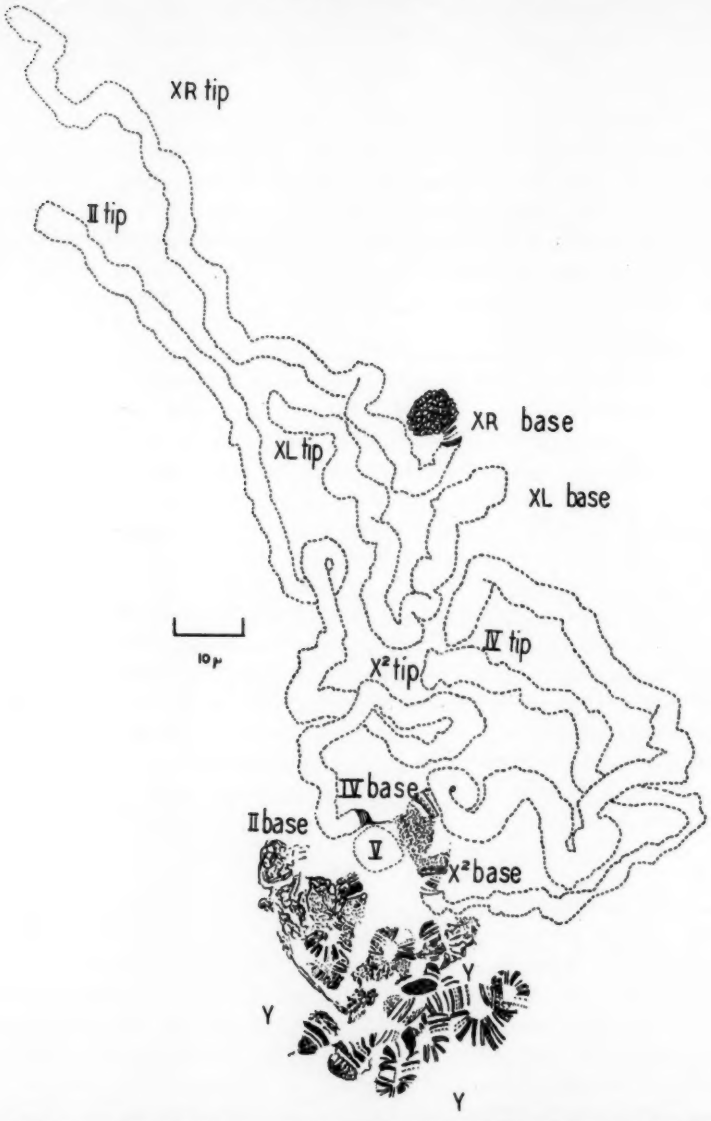

FIGURE 1.—Camera-lucida drawing of a complete salivary gland nucleus of a male of *Drosophila miranda*. The banding of XL, XR, II, X^2, IV, and V has been omitted except for a few bands at the bases of XR, II, X^2, and IV. With this exception, all the banded material shown belongs to the Y chromosome.

well-known behavior of rings in Datura, Oenothera, and Triticum. But since DOBZHANSKY (1935) found no trivalent association, it would be necessary to suppose that the association between Y and X^2 was either obliterated or in some way obscured in the stage at which DOBZHANSKY's observations were made.

If Y and X^2 conjugated in meiosis, it might be expected that pairing between these chromosomes would be seen in the salivary gland nuclei. No such pairing has been seen in the many nuclei examined. While this direct evidence of homology is therefore lacking, the lack of it, as will be seen later, does not necessarily constitute evidence against homology. In order to decide the question we must seek indirect evidence. To this end, and for the sake of the bearing it has on the question of directed segregation, as well as for its intrinsic interest, an account of the genetic studies which have been made on *miranda* will now be presented.

METHODS

Drosophila miranda is not an easy species to breed. The life-cycle requires nearly a month, if one uses the individuals whose development has been most rapid. Some individuals do not emerge until more than 36 days after oviposition. As a result the food is apt to become progressively worse in experiments in which an effort is being made to secure complete counts. Since the males develop more slowly than the females, they are exposed to less favorable conditions; this accounts for part of the excess of females over males (DOBZHANSKY 1935). As adults the males are smaller and shorter-lived than the females. It seems probable that male and female zygotes are formed with equal frequency, since from a given number of eggs one does not obtain more than half that number of females (DOBZHANSKY 1935). The tendency of these large and sluggish flies to become stuck in the food has been overcome by covering the food with a very light sprinkling of sawdust, no more than will adhere when the bottle is shaken upside down. It is even possible to drop etherized flies onto a food surface so prepared without their becoming stuck.

SEX-LINKED MUTANTS

Truncate (*Trn*) is almost completely dominant. It is often difficult, sometimes impossible, to tell whether a female is homozygous or heterozygous. The character is somewhat more extreme in the male. The marginal vein at the wing-tip is usually gone, though a scattering of hairs is sometimes left. The wing is shorter, and the veins, especially III, are still shorter, so that they do not reach even to the new margin. The scutellum is broad and blunt, and the bristles on it are usually reduplicated and irregular. There is a rough patch in the eye at the center of the anterior

margin and extending backwards from there. The viability of the heterozygous females is excellent, that of the males poor. The character is inherited as a typical sex-linked dominant.

Since *Trn* arose as a result of X-ray treatment, the homozygous Truncate stock was crossed with wild type and the salivary gland chromosomes of the resulting females were examined in order to find out whether any chromosomal aberration was present. It was found that these females were heterozygous for a very small inversion in X^2, about one-fifth of the way from the tip. Males from the same cross had no such heterozygous inversion figure in the X^2, which confirms DOBZHANSKY'S conclusion that X^2 is unpaired in males. While it seemed reasonable to assume that the loci of Truncate and of the inversion were the same, the matter was tested by following them genetically and cytologically in successive generations of outcrossing. It was found that they did not separate, even though crossing over took place nearby. Truncate is therefore in X^2.

Crossveinless, a recessive, closely resembles *cv* of *melanogaster* in appearance. Its viability is poor. Males carrying both *Trn* and *cv* die, many of them in a late pupal stage. Two have been obtained as adults. Both died without issue soon after emergence. Females from the cross of *cv* by wild type are heterozygous for a very small inversion in XL about one-third of the way from the tip, though this fact was not discovered until after *cv* had been placed in X by another method (cf. Crosses with *pseudoobscura*). Very probably *cv* is located at or close to the inversion.

Plexus, a recessive, causes extra veins in the wing, especially extra crossveins in the tip of the submarginal cell. The character is less pronounced in males than in females, almost, but not quite, overlapping normal. I have not been able to detect any cytological abnormality in connection with *px*. Unpublished data of DUNCAN show that *px* is linked to *Trn*, giving about seven percent recombination with it. This is in accord with the results of the present study. Two independent experiments have shown that *px* and *cv* assort at random. One, involving 263 individuals, gave 46 percent recombination. Another, involving 950, gave 49.5 percent.

Vermilion (*v*) occurred spontaneously as a *v cv px* male in a linkage experiment involving *Trn*, *cv*, and *px*. It has never separated from *cv*, although more than 1,000 non-crossover individuals have been obtained. This is a reason for thinking that the small heterozygous inversion seen in females heterozygous for *cv* is at or close to the locus of *cv*.

Javelin (*jv*) makes the bristles slender and less curved than normal. They are often broken off near the tip. It closely resembles slender of *pseudoobscura* (XR) and javelin of *melanogaster* (III L). Javelin occurred spontaneously in the Smoky 14 stock. It was crossed to *Trn*, and a recombination value of 49.0 percent, based on 149 individuals, was obtained.

With *cv*, it gave 51.2 percent crossing over (506 individuals). As will be shown below, it is probably in X^1, and since it shows no linkage with *cv* (XL) it is probably in XR.

AUTOSOMAL MUTANTS

The only autosomal mutants known are the Smokys, *Sm 3, 8, 9, 10, 14*. The unpublished work of DUNCAN indicates that these five are dominants, lethal when homozygous, and allelic to each other and to *Sm 11*. Smoky of *pseudoobscura*, which the character closely resembles, is situated close to the base of II.

PATROCLINOUS MUTANTS

The patroclinous characters, of which two are known, are transmitted by a male to all his sons and to none of his daughters. Thus in a true-breeding stock, the character is shown by all the males, and by none of the females. In outcrosses, the females do not transmit the character to their descendants of the first nor second generation, while the males transmit it to all their sons. In short, the distribution of the character follows that of the Y chromosome.

One of the two characters with this behavior is *Sm 11*, which phenotypically resembles the autosomal Smokys. Slightly under 2,000 flies of this stock have been systematically examined, and a great many more have been looked at in the course of breeding work, without a Smoky female or a non-Smoky male being found.

A study of the salivary gland chromosomes of a male from the *Sm 11* stock showed that the basal part of II was frequently unsynapsed, one member of the pair being attached to an unidentified heterochromatic body. A careful study has not been made, but it appears that a II-Y translocation has occurred.

The other patroclinous character, Angles, was recently obtained in an X-ray experiment. Typically the anal angles of the wings are clipped off. However, the character is rather variable. The clipping may extend irregularly all along the posterior margin of the wing. On the other hand there may be only a slight disturbance of the marginal hairs at the angle. It is not uncommon for the character to show in one wing only, and one occasionally finds males of this stock which do not show the character at all. Such males, however, so far as tested, have always produced a high percentage of Angles sons when outcrossed.

From a study of the salivary gland chromosomes of the males of the Angles stock it is clear that they carry a reciprocal translocation between the euchromatic part of IV and one of the euchromatic sections of Y. Chromosome IV is broken about one-third of the way from the free end. The euchromatic section of Y is broken about in the middle.

In neither *Sm 11* nor *An*, so far as I can see, are the mitotic chromosomes different from normal. Both these mutations, *Sm 11* and *An*, have arisen in connection with translocations between autosomes and the Y. In one of them, *Sm 11*, it is clear that the mutated gene is of autosomal origin. It is not possible to say whether the Angles gene is in the material of IV or of Y.

Both these patroclinous genes are dominant. This is not a matter of chance. On the contrary it is important to recognize that a recessive gene transmitted in a similar fashion would produce no effect at all, and would therefore not be detected. This would be true whether the recessive were in the Y or in an autosome which was involved in a Y-translocation, since in neither case could the gene become homozygous. Even though the gene were in the autosome at some distance from the point of breakage, the gene would not be able to get into the unaltered autosome, since there is no crossing over in the male. This point will be discussed further.

SUMMARY OF EVIDENCE FROM GENETIC STUDIES

Are zygotes formed in *miranda* whose chromosomal constitutions are different from those which are typical for the species? The following facts should be considered. In the first place, adults are of two phenotypic classes, normal males and normal females. There are no classes of abnormal or intersexual individuals produced, even in small numbers. Hence, if exceptional zygotes are formed whose chromosome constitution is other than normal male or female, such zygotes either die before reaching the adult stage or give rise to adults of normal appearance. From DOBZHANSKY's (1935) data on mortality it is clear that the number of zygotes that die cannot be large. The number of exceptional zygotes that survive is not large either, or some would have been detected cytologically. But cytological study has been made of only a few individuals, relatively; in order to have large enough numbers on which to base our conclusions, we must use the results of genetic study.

Since it seems clear that the eggs are all of one sort, X^1X^2A, let us consider what sort of segregation in the male would be required for the production of zygotes of exceptional constitution. The male is X^1YX^2 2A, where A represents a set of autosomes, II, IV, and V. Zygotes haploid or triploid for II or IV will be inviable. Whether haplo- and triplo-V individuals are viable is of no present interest. Let us therefore neglect the autosomes and consider only the segregation of X^1, Y, and X^2. With respect to these three chromosomes there are 2^3 or 8 possible types of sperms: (see table 1).

In the first place, types 5 and 6 are the regular male and female producing sperm respectively.

The finding that the Y contains a large amount of euchromatic material

probably homologous to X^2 leads us to expect that sperm of the sorts 1, 2, 3, and 4 will give rise to inviable zygotes, since these will carry either a deficiency (1, 3) or a duplication (2, 4) for the material of an entire chromosome. If such sperm are formed, the zygotes from them certainly die, for there is genetic evidence that they do not give rise to adults (table 1). But conversely, their failure to give rise to viable zygotes is not evidence against the production of these types of sperm; indeed, they may be produced in numbers up to the limits set by zygotic mortality, or in larger numbers if the unlikely assumption of gametic selection be made.

TABLE 1

A summary of the evidence from breeding tests within D. miranda showing that no appreciable number of individuals having exceptional sex-chromosome constitution survives to the adult stage. The table is self-explanatory, except for the two entries referring to Sm 11, which involve a few obvious and reasonable assumptions.

SPERM OF TYPE	DO NOT PRODUCE ADULT MALES BECAUSE:	DO NOT PRODUCE ADULT FEMALES BECAUSE:
(1) A	$+\,♀ \times Sm11\,♂$ produce no $+^{Sm11}\,♂$	*cv* ♀ × + ♂ produce no *cv* ♀
(2) X^1YX^2 A	*cv* ♀ × + ♂ produce no $+^{cv}$ ♂	$+\,♀ \times Sm11\,♂$ produce no *Sm11* ♀
(3) X^1 A	*cv* ♀ × + ♂ produce no $+^{cv}$ ♂	$+\,♀ \times Trn\,♂$ produce no $+^{Trn}$ ♀
(4) YX^2 A	$+\,♀ \times Trn\,♂$ produce no *Trn* ♂	*cv* ♀ × + ♂ produce no *cv* ♀
(5) Y A	—	*cv* ♀ × + ♂ produce no *cv* ♀
(6) X^1X^2 A	*cv* ♀ × + ♂ produce no $+^{cv}$ ♂	—
(7) X^2 A	$+\,♀ \times Trn\,♂$ produce no *Trn* ♂	*cv* ♀ × + ♂ produce no *cv* ♀
(8) X^1Y A	*cv* ♀ × + ♂ produce no $+^{cv}$ ♂	$+\,♀ \times Trn\,♂$ produce no $+^{Trn}$ ♀

The sperm of types 7 and 8 remain to be considered; their behavior is of particular interest. For if, as we suppose, the euchromatic material of Y is substantially equivalent to X^2, while the heterochromatic material resembles the Y of *pseudoobscura* in that deficiency and excess of it have no effect on viability, then we would expect that sperms of types 7 and 8, if formed, would produce zygotes of normal viability. Genetic evidence (table 1), and especially the inheritance of *Sm 11*, show that the expected classes of adults do not appear; from this we might conclude that such sperm are not formed. Such a conclusion would be false, as will shortly appear; there is definite evidence that sperm of type 7 are produced with a frequency of at least 1 in 268. Since the zygotes coming from sperm of type 7 are not viable, while those from type 5 are, it follows that X^2 and Y are at any rate not sufficiently alike to be interchangeable. This fact will be discussed below, but first the evidence will be presented which demonstrates the production of X^2 A sperm.

HYBRIDS WITH PSEUDOOBSCURA

DOBZHANSKY (1937) has described in detail his observations on crosses between *miranda* and *pseudoobscura*. Offspring can be obtained from each

of the two reciprocal crosses. The female hybrids from both crosses are normal in appearance, but were in DOBZHANSKY's experiments completely sterile. It now appears that they are not invariably so. STURTEVANT (unpublished), using a somewhat different method, found it possible to obtain hybrid females which were slightly fertile. This result has also been obtained by DOBZHANSKY (unpublished) in a repetition and extension of his earlier experiments. He has found that the fertility of the hybrid females varies from very slight to almost nil, depending on the geographic races used in making the cross. Some of the most fertile female hybrids so far obtained have come from crosses of the Mt. Whitney strain of *miranda* with standard laboratory stocks of *pseudoobscura;* these hybrid females have about one percent of the fertility of females of the pure races. There will be presented below the results which have been obtained by using such females for backcrossing experiments. A simple F_2 cannot be raised since the male hybrids are totally sterile regardless of how the cross is made.

With respect to the production of hybrid males, the two reciprocal crosses give, as DOBZHANSKY (1935, 1937) has shown, entirely different results. *Miranda* females crossed to *pseudoobscura* males give male hybrids which approach the female hybrids in point of numbers, but unlike them are morphologically abnormal. It seems beyond question that DOBZHANSKY (1937) is right in concluding that they arise from the regular male-producing *pseudoobscura* sperm, the deficiency in their numbers compared with females being due to zygotic mortality. Summed data from several of my cultures show 215 males to 336 females or 32 males per 50 females.

This cross offers an opportunity to test whether a sex-linked recessive in *miranda* is in X^1 or in X^2. If the recessive is in X^1, then when a homozygous mutant *miranda* female is crossed to a wild type *pseudoobscura* male, the character should appear in the hybrid males, since most of the material of X^1 is in X of *pseudoobscura*, and since these hybrid males come from the Y-bearing sperm which lack the X. If the recessive is in X^2, the hybrid males should not show the character, since they receive the *pseudoobscura* III. The test has been applied to *cv*, *jv*, and *px*. The effects of *cv* and *jv* showed in the hybrid males, those of *px* did not. Hence *cv* and *jv* are in X^1, *px* in X^2, an inference which is supported by several other pieces of evidence. It was not necessary to apply this test to *v*, for a cross to *v* of *pseudoobscura* (XL) showed the two to be allelic. A cross was also made by DUNCAN between *cv* (*miranda*) and *cv* (*pseudoobscura*, III) a mutant which superficially resembles it; the female hybrids were wild type.

Most interesting of all, *px* was tested against *px* of *pseudoobscura* and

found to be allelic. This is striking because *px* (*miranda*) is sex-linked while *px* (*pseudoobscura*) is in an autosome (III). Thus *miranda* and *pseudoobscura* stand in the same relation with respect to *px* as *pseudoobscura* and *melanogaster* with respect to sepia, javelin, veinlet, and others. The case of *px* is discussed again in a later section.

EXCEPTIONAL HYBRID MALES

On the other hand, the reciprocal cross, *pseudoobscura* female by *miranda* male, (DOBZHANSKY 1935, 1937) produces only one male to about 268 females. Unlike the males from the other cross, these males, though small, are well-proportioned. For this reason DOBZHANSKY (1937) thought it unlikely that they came from the regular male-producing *miranda* sperm, representing the rare survivors of a class originally as numerous as the females. Having excluded the possibility that they came from no-X eggs by the observation that they showed sex-linked recessives from the mother, he sought to determine whether these exceptional males received an X^2 from the father, using the following method. He reasoned that the *miranda* male was haploid for the material of III, carrying it only in the unpaired X^2, and hence if a *pseudoobscura* female homozygous for a third chromosome recessive were mated to a wild type *miranda* male, the presence or absence of an X^2 in the hybrid male would be indicated by the suppression or appearance respectively of the recessive from the mother. He tried the experiment using orange and purple; both were suppressed in the hybrid males. From this he concluded that these males were produced by sperm carrying a set of autosomes, X^2, and probably also the Y.

The finding that the Y contained a large amount of euchromatin presumably homologous to III seemed to reopen the question. In the first place, it seemed doubtful that the hybrid males could get both X^2 and Y from the father, for these added to the III from the mother would make them carry a duplication for an entire chromosome. But what was worse, the *miranda* Y might carry the normal alleles of orange and purple. Without this being disproven, DOBZHANSKY'S experiment remained inconclusive: the hybrid males might come from normal male producing sperm, YA. While their rarity argued against this, the rare survival of *Trn cv* males showed that such a thing was not impossible. Fortunately it was easy to test the matter. The objections which applied to orange and purple did not apply to plexus, for the *miranda* Y was known not to contain its wild type allele. If it did, *px* in *miranda* would be sex-linked like bobbed, rather than sex-linked like white.

Pseudoobscura females homozygous for *px* were crossed to wild type *miranda* males. The progeny consisted of 1,466 not-*px* females and six not-*px* males. This showed that DOBZHANSKY'S conclusion was correct;

the hybrid males do receive X^2 from the father. This was confirmed by crossing wild type *pseudoobscura* females to *Trn miranda* males. A progeny of 2,699 females and three males was obtained. Two of them showed *Trn*, as expected. The third was unfortunately not classified for the character. The low frequency of males is probably due to the effect of *Trn* on viability.

The question remained whether or not these males received the Y as well as the X^2 from the father. This was decided in the negative by a cross of wild type *pseudoobscura* females to *Sm 11* males. The progeny consisted of 404+ females and three+ males. It might be mentioned that this is the only way in which a non-*Sm 11* son has ever been obtained from a *Sm 11* father.

Genetic evidence thus shows clearly that the exceptional males come from X^2 A sperm. This has also been shown cytologically. Two exceptional males were obtained in the larval stage and ganglion preparations made. They both had six rods and a V. In one the pair of dots was clearly seen.

The most important fact which emerges from these studies is that the exceptional hybrid males do not result from the failure of the mechanism of determinate disjunction, as suggested by DOBZHANSKY'S (1937) figure 5, but from primary non-disjunction of X^1 and Y chromosomes. The frequency of this phenomenon is of the order of magnitude of the frequency of exceptional males. Combining DOBZHANSKY'S (1937) data with mine, a figure of 78 males: 20,819 females is obtained, or 1:268. This figure does not include the experiments involving *Trn*, which gave a rather low value, nor an experiment in which Angles (IV-Y Translocation) males were used, which gave a rather high value of 10 males to 806 females.

Primary nondisjunction could also give rise to another class of sperm, type 8 of table 1, having X^1, Y, and A. Such sperm, if formed with a corresponding frequency, do not give rise to viable zygotes when they fertilize *miranda* eggs, (table 1), nor when they fertilize *pseudoobscura* eggs, for none were found among 4,569 hybrids from which such individuals could have been distinguished genetically, had they occurred. In fact there is no evidence at all for the formation of such sperm, though it does not follow that they are not formed. The reader will, however, recall that primary non-disjunction in the female of *melanogaster* gives rise much more frequently to no-X eggs than to 2X eggs. Loss of the heterochromosome bivalent by its failing to divide and remaining on the equatorial plate may explain both cases.

The above-mentioned exceptional males which were obtained in the larval stage were found in the course of an attempt to study zygote mortality in the cross of *pseudoobscura* females by *miranda* males. The females carried eosin, a sex-linked recessive which not only lightens the eye-color,

but also makes colorless the Malpighian tubules, which normally are yellow. They were crossed to *miranda* males of the Olympic 1 stock, and their eggs collected. Most of them produced no viable eggs while under observation, many no eggs at all. Probably most of them were never inseminated. In all, 90 larvae were obtained; these were classified for Malpighian tubule color. Yellow tubules were present in 77 of them, 11 died or were lost before they could be classified, and two had colorless tubules; these are the two exceptional males mentioned above.

In computing zygotic mortality, we must bear in mind that failure of an egg to hatch in this species cross may be due merely to failure of fertilization. If we therefore exclude those daily layings of eggs of which none or only a few hatched, we will get a better estimate of the viability of hybrid zygotes. Of the 90 larvae, two-thirds were obtained from four daily layings of 105, 5, 12 and 19 eggs; total 141. Of these, 41, 4, 6, and 9 respectively hatched; total 60. This shows that the viability of the hybrid females is good. A more significant fact shown by this experiment is that the zygotes resulting from the union of regular male-producing sperm of *miranda* with eggs of *pseudoobscura* die at a very early stage. Of the 77 to 88 of them expected, not more than 11, and probably none at all, hatched from the egg. Certainly they do not survive to the second larval instar. This unexpected finding serves to emphasize that X^2 and Y are decidedly not interchangeable. An X^2 A sperm produces an inviable zygote if it fertilizes a *miranda* egg; a YA sperm produces one which lives. With a *pseudoobscura* egg the reverse happens, and the inviable zygote dies at a surprisingly early stage.

COMPOSITION OF THE MIRANDA Y

We have already seen another respect in which the *miranda* Y differs from the X^2, namely, that it has either a recessive allele or a deficiency at the *px* locus. If other recessives in X^2 were available, it would be possible to test whether or not the Y carried their wild-type alleles by simply seeing whether the character showed in males. Unfortunately *px* is the only X^2 recessive thus far found. Many recessives are available in III of *pseudoobscura*, but it is not possible to determine whether their wild type alleles are present in the *miranda* Y simply by crossing to a *miranda* male, since the few males produced receive the X^2, and not the Y, from the father. I have tried to make use of the slight fertility of the hybrid females in order to overcome this difficulty. By backcrossing to *Sm 11* males for several generations it has been possible to obtain a male with the *miranda* Y and a part of the *pseudoobscura* III carrying orange and Blade. The male's eyes were orange; Blade appeared as it does when heterozygous. From this it may be concluded that the miranda Y lacks the wild type

allele of orange, as it does of *px*, but very probably carries that of Blade. These experiments are still in progress, and may furnish similar information about other *pseudoobscura* genes.

DISCUSSION

The purpose of this discussion is twofold; first, to present and criticize the view of the construction, operation, and evolution of the sex-determining mechanism of *Drosophila miranda* to which the observations reported in this paper have led, and, second, to suggest some bearings which they may have on the evolution of sex-determining mechanisms in general.

It seems likely that the first step in the origin of the *miranda* Y was the

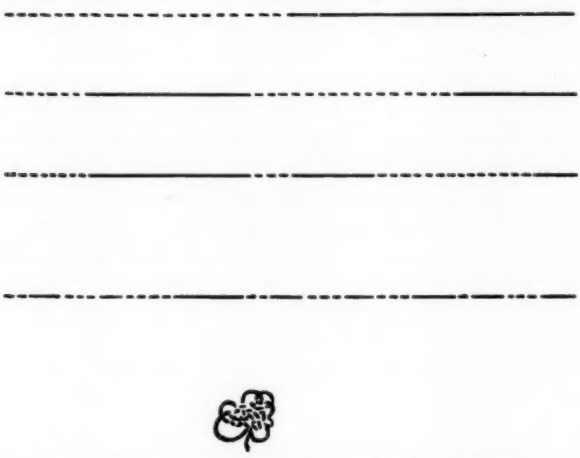

FIGURE 2.—Heterochromatin dotted line, euchromatin solid line. Depicted are the results of fusion of Y and III, and of one, two, and several subsequent inversions. At the bottom, the configuration which would be produced by the non-specific association of heterochromatin, and which would therefore be expected in the salivary gland nuclei.

fusion of III and Y at the spindle attachment region. Had the Y possessed two arms, this might have involved the loss of one of them. However, wide variations in the size of the Y are known to exist in different strains of *pseudoobscura* today, without apparent effect on the fertility of the male. Perhaps a rod-shaped Y may have been present in the male in which the fusion took place. After the two chromosomes had become one, extensive rearrangement must have taken place within it, in the course of which the euchromatic material came to be in the form of many short segments inserted at various points in the heterochromatin. Of course, one might equally well think of the heterochromatin being scattered through the euchromatin. By way of example, figure 2 shows how successive inversions might have led to such a condition.

Such rearrangements would not have resulted in any difficulties connected with crossing over, since the chromosome would have been present only in males. How the pairing relations between Y and III would have been affected is less clear. It is hoped that DR. KOLLER'S current investigations may shed light on this question. In the salivary gland nuclei, the non-specific affinity of heterochromatin (BAUER 1936) would lead to the association of all the heterochromatic regions in the Y with each other. Such is my interpretation of the condition seen in figure 1. The extensive rearrangement of the euchromatic material in Y, perhaps also its position in the nucleus, would result in the observed rarity or absence of pairing between it and X^2. This observation, and other considerations as well, suggest that meiotic pairing between X^2 and Y, if it occurs, may involve a heterochromatic region.

This view of the sex-determining mechanism of *Drosophila miranda* is, I believe, consistent with all known facts concerning it. DARLINGTON (1936) has put forward a different theory which is at variance with the facts available at the time of its publication (DOBZHANSKY 1935) as well as with those that have appeared since (DOBZHANSKY & TAN 1936, DOBZHANSKY 1937, MACKNIGHT 1938, and the present paper). Strangest of all, it was because of DARLINGTON'S idea that directed segregation "almost uproots genetics" that he felt driven to put forward an alternative theory. He apparently did not realize that his own theory also demanded directed segregation, since whatever he might assume about the internal composition of Y and X, the fact remained that they were visibly different, and that the Y occurred only in males. He apparently also did not realize that a well-authenticated case of the directed segregation of chromosomes not in contact at metaphase had long been on record. In *Gryllotalpa borealis* there are present an unequal bivalent and a univalent (BAUMGARTNER 1911). (These observations are not to be confused with the dubious and in any case irrelevant studies made by VOINOV (1914, but compare 1912) on a different species, *G. vulgaris*.) The univalent and the larger member of the bivalent always go to the same pole (PAYNE 1912, 1916). The other pole receives the smaller member of the bivalent, which we may call Y. Sperm receiving Y give rise to males. The sperm which receive the two X chromosomes (we may call them X^1 and X^2) give rise to females. However, it is interesting, and perhaps important, that one of PAYNE'S (1916) figures shows a connection between the unequal bivalent and the univalent. An examination of the prophases might prove fruitful, especially in view of the work of CAROTHERS (1913) who found a bivalent which divided unequally in the first meiotic divison, after having been associated with the accessory in prophase. At metaphase the bivalent was no longer associated with the accessory, and disjoined at random with

respect to it. But the unequal bivalent was present in all the twenty males examined. CAROTHERS assumed that the female also carried the unequal bivalent and that strict selective fertilization took place. I would prefer to suppose that zygotic mortality was the explanation.

Be that as it may, it is clear that in *miranda* both directed segregation and zygotic mortality are involved. Directed segregation makes exceptional zygotes rare. When they occur, zygotic mortality eliminates them.

We have formed a picture of the sex-chromosome mechanism as it exists in *miranda* today, and of the stages through which it must have gone in the past, since the time when it diverged from the *pseudoobscura* type. If we have said nothing about the possible causes of its divergence and subsequent evolution, it is for reasons not far to seek. But where information is lacking, it is tempting to speculate. There is present in wild populations of *pseudoobscura* a gene, "sex-ratio" (STURTEVANT and DOBZHANSKY 1936) which, by virtue of its effect on its own distribution at meiosis, tends to displace its normal allele. It is sex-linked, and a male carrying it produces almost all daughters, so that a population homozygous for it would die out. But if a III-Y fusion chanced to occur and to spread through a small population, that population would then be protected against such a danger, for "sex-ratio" would have much less tendency to spread in it. This follows from the fact that the Y is eliminated during the maturation divisions of the "sex-ratio" male. If a III-Y fusion were present, the fertility of the male would be halved. Again, a population carrying a III-Y fusion might be at an advantage in that half its members would benefit from whatever heterosis resulted from III being kept heterozygous.

It is necessary to seek some reason for the spread of this fusion, for there can be no doubt that it is, and has been from the first, responsible for a certain amount of zygote mortality. But no zygote mortality need have occurred during the transition from a *melanogaster*-like condition to that found in *pseudoobscura*, except perhaps at first. Let us suppose that in the common ancestor of *melanogaster* and *pseudoobscura* the material of III L (*melanogaster*) (=XR *pseudoobscura*) was in the form of paired rods. In the *melanogaster* line of descent it would undergo no change except to become attached to III R. In the *pseudoobscura* line of descent we would suppose that a fusion of one member of the pair with Y took place, and, perhaps simultaneously, perhaps not, a fusion of the other member of the pair with X. (Similar fusions have actually been obtained in Drosophila by PAINTER and STONE (1935).) The *pseudoobscura* condition would thus be reached, with the very important exception that the male, instead of being haploid for the material of III L (=XR), would carry it in duplicate, once in the X and once in the Y. (It should be mentioned here that with

this point in view I carefully examined the salivary gland chromosomes of a *pseudoobscura* male of the Oaxaca 4 race, in which the largest Y occurs. I could find no euchromatin in the Y.)

Thus we arrive at what is perhaps the most interesting question of all. Can we suppose that euchromatic material in the Y would degenerate and ultimately disappear as a result of its being kept heterozygous? Such and idea was propounded by MULLER and STURTEVANT (MULLER 1914) to account for the absence of genes in the Y, and has since been elaborated by MULLER (1918), and, among others, by HALDANE (1933). Opposed to this view is FISHER (1935), who believes he has shown by mathematical considerations that no accumulation of deficiencies, lethals or deleterious genes, even though recessive, could take place; that it would be effectively prevented by the occasional presence of a mutant gene at the same locus in the homologue. However, FISHER'S mathematical treatment is based on a number of premises. These are not all correct, and one might expect the same to be true of his conclusions. The present study has shown that deleterious genes (deficiences or mutant alleles at the *or* and *px* loci) have in fact become established in the material of III since it became a part of Y. The time required for this process has been so short, geologically speaking, as scarcely to permit *pseudoobscura* and *miranda* to become visibly different in external morphology. I think it not at all unreasonable to suppose that *pseudoobscura* and *melanogaster*, which are very different externally, have been separated long enough for an entire chromosome-arm, attached to the Y, to degenerate and disappear.

To be sure, the *or* and *px* loci are the only ones for which the *miranda* Y is known not to carry a normal allele. But they are two of the three loci which it has so far been possible to test. There can be no doubt that the material of III in the Y has been greatly altered. How different the Y is from X^2 in total effect is well shown by the fact that hybrid males carrying X^2 survive to the adult stage, while those carrying Y, the other chromosomes being the same, die before hatching from the egg. On the other hand, individuals of pure *miranda* having the male set, except for Y being replaced by X^2, do not live to be adults, but die in an immature stage, though precisely which stage it is not possible to say.

I believe that positive selective action has aided passive mutation in making Y and X^2 different. $X^1{}_2X^2{}_2A$ individuals would have been sterile from the time when the Y-III fusion originated, since they would have lacked the Y (which in *pseudoobscura* as in *melanogaster* carries factors necessary for the normal development of the spermatids into spermatozoa). Such sterile males would have been disadvantageous to their blood relations. Since Drosophila is not a social animal, sterile individuals are entirely worthless; indeed they compete with fertile individuals for mates

and food. Consequently some geographic races, some local populations of what has since become *Drosophila miranda* would have been at a selective advantage if they possessed such a gene complex that $X^1{}_2X^2{}_2A$ males were weak or short-lived as adults, or survived to that stage less often. Such selective action would continue till $X^1{}_2X^2{}_2A$ zygotes did not hatch from the egg. Since there is no competition between unhatched zygotes, selection would not tend to make zygotes die at a still earlier stage, unless perhaps by way of insuring against even their occasional survival. I shall attempt to find out how far this process has actually gone. Of course there would be a concurrent selection of the same nature against the *production* of $X^1{}_2X^2{}_2A$ zygotes, that is, for regular segregation in the male.

Probably a selection of similar nature would act to make hybrids between *miranda* and *pseudoobscura* sterile or inviable (STURTEVANT 1938). DOBZHANSKY and TAN (1937) have suggested one possible reason why the hybrids of the heterozygous sex are, as HALDANE (1933) had pointed out, usually the less fertile and the less viable. In the present connection I should only like to add that, in the cross of a *pseudoobscura* female by *miranda* male, selection against the survival of X III A (*pseudoobscura*) YA (*miranda*) zygotes would be about 267 times as strong as that against the survival of X III A (*pseudoobscura*) X^2A (*miranda*) individuals.

It is only on the recent evolution of the *miranda* sex mechanism that this comparative study has cast any light. What the series of changes may have been which led to the condition present in the common ancestor of *miranda*, *pseudoobscura*, and *melanogaster* is a question which must be answered by further study. Within the genus or near it there are surely species by the investigation of whose sex-mechanism this question could be at least partially answered, and a material contribution to our understanding of the evolution of sex-determination thereby made.

SUMMARY

1. *Drosophila miranda* and *Drosophila pseudoobscura* are two closely related species whose most striking difference is in the structure of their sex-determining mechanisms. The nature of this difference is elucidated in the present paper.

2. An attempt is made to reconstruct the recent evolution of the *miranda* mechanism. It is shown that degenerative changes have been retained in chromosomal material kept heterozygous by being attached to the Y.

3. On the basis of this study a better understanding of other variations in the sex-determining mechanism within the genus Drosophila is possible. Some bearings of this study on the general question of the evolution of sex-determining mechanisms are discussed.

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GENETICS OF PARAMECIUM BURSARIA. I. MATING TYPES AND GROUPS, THEIR INTERRELATIONS AND DISTRIBUTION; MATING BEHAVIOR AND SELF STERILITY*

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Received November 14, 1938

PARAMECIUM *bursaria* is one of the most widely distributed of the species of Paramecium, though it does not as a rule occur in such large numbers in a given region as does sometimes *Paramecium caudatum*. It occurs commonly amid growing vegetation in the quiet parts of pools, ponds or streams. It does not flourish in regions in which the water is foul or there is much decaying vegetation.

The species is commonly at once recognizable by the algal cells which it contains, which give it a green color. The algal cells are usually packed closely, but at times they are few and scattered or perhaps even non-existent, so that the animals are nearly white. Such white individuals may readily be produced by allowing the animals to multiply rapidly in a rich culture medium. The ciliates then multiply more rapidly than do the algae, so that the animals are left with almost none of the algal cells.

Figures and descriptions of this species in comparison with others are given by WENRICH (1928), KALMUS (1931), and KAHL (1931). Many diverse biotypes occur, which may differ greatly in form, size and physiological characteristics. The typical form is more flattened, "slipper shaped," than in *Paramecium caudatum* or *aurelia*. The flattened body is slightly curved in a segment of a flat spiral, the broad oral groove extending on one surface from the anterior end to about the middle of the body. The anterior tip projects forward, to the right, and a little downward (toward the oral surface); in very pronounced cases this gives almost the appearance of a hook. Typical forms and sizes for certain biotypes are shown in outline in figure 7 on page 225. As there illustrated, the length ranges from about 100 microns (in the clone l figure 7, 2) to about 160 microns or a little more, in a clone of the type E from California (figure 7, 12).

Paramecium bursaria has been much employed in genetic and physiological investigations. The earlier studies on it are resumed by MAUPAS (1889, pp. 228–238), who himself studied the nuclear processes in conjuga-

* A part of the cost of the illustrations has been defrayed by the Galton and Mendel Memorial Fund.

[1] This work has been aided by a grant from the Carnegie Corporation. For efficient assistance with the cultures and experiments the writer is indebted to his assistant, DR. ELIZABETH S. KIRKWOOD, and for shorter periods to DR. N. HIGGINBOTHAM and DR. L. B. SHETTLES.

tion. The cytology of conjugation was again extensively studied by HAMBURGER (1904).

CULTURAL CONDITIONS

Paramecium bursaria flourishes and multiplies rapidly in the algal-bacterial culture medium of RAFFEL (1930). Powdered desiccated lettuce, 1.5 grams, is boiled for five minutes in a liter of distilled water; the fluid is filtered and distributed into pyrex flasks containing an excess of $CaCO_3$; the flasks are stoppered with cotton and autoclaved. This constitutes the stock fluid. The culture medium for actual use is prepared from this as follows. To 20 cc of the filtered fluid is added one 1 mm loop of *Flavobacterium brunneum* taken from a three- to five-day old agar slant; also three 2 mm loops of the alga *Stichococcus bacillaris* from an 18-day old agar slant. In this medium the rapidly dividing animals become large and plump. They flourish well in the same fluid diluted to various strengths with water; also in hay infusion. For keeping many clones for long periods, it is convenient to use a dilute hay infusion, made by boiling one gram of timothy hay (*Phleum pratense*) in 800 cc of water for ten minutes. This may advantageously be infected at the beginning with a small quantity of the algal-bacterial medium above described. On becoming established in a vial, finger bowl, or other small vessel in a small quantity of this dilute hay infusion, together with three or four bits of boiled hay an inch or less in length, cultures of the organism may flourish for months, without further attention, except that water lost by evaporation must be supplied at intervals. For the genetic experiments, sometimes more than a thousand such cultures were kept in progress for long periods.

PRINGSHEIM (1915), ERDMANN (1927) and LOEFER (1936) have cultured this species in bacteria-free mediums. Such culture was not attempted for the very numerous stocks required in the present investigation.

AGGLUTINATION AND MATING

The key to the genetics of the species is given by the peculiar relations in mating. Individuals belonging to the same clone usually do not mate ("self sterility"); exceptions to this are dealt with later. Individuals of diverse clones that are not of the same mating type (but yet belong to the same "group"), agglutinate and conjugate when mixed (provided they are mature and in the proper physiological condition) (figure 1).

The phenomena of agglutination or clumping, with later formation of pairs, when diverse mating types are mixed, are spectacular. In the typical case, when many individuals of the two types are placed together in a small amount of water, the individuals are seen to begin at once to become clumped into dense groups. The groups rapidly increase in size, so that

in a few minutes clumps containing hundreds of individuals may be produced. Later the clumps begin to disintegrate, and many of the components as they separate from the mass are seen to be united in pairs by their oral surfaces. Such pairs continue union till the individuals have

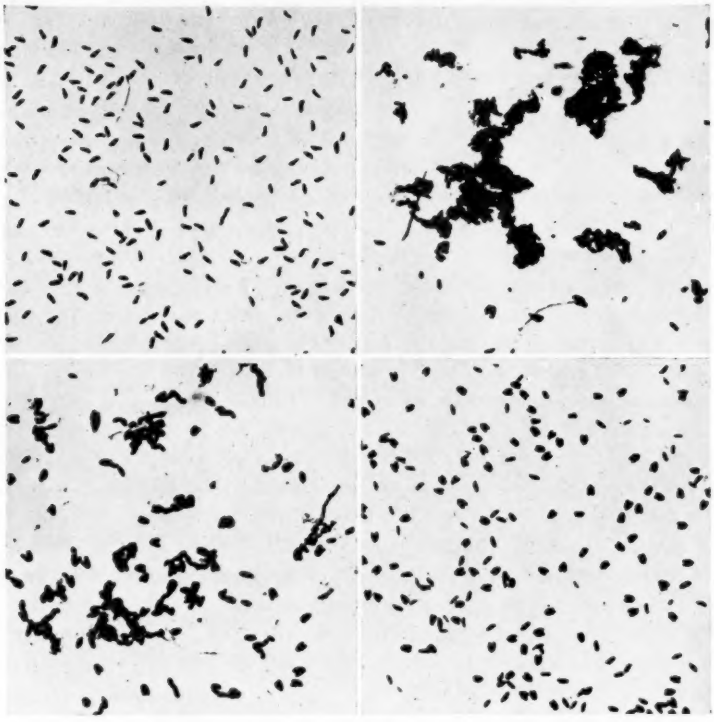

FIGURE 1.—Photographs of the mating behavior in *Paramecium bursaria*.
Upper left, single mating type (C); the individuals scattered singly.
Upper right, mixture of two types (P and Q), six minutes after the two were mixed. The individuals agglutinated in large masses.
Lower left, mixture of two types (P and Q) about five hours later. The large masses have broken down into small masses, chains (center above) and pairs.
Lower right, mixture of two types (P and Q) 24 hours later. Most of the individuals in pairs.

undergone the characteristic changes that accompany conjugation, including the exchange of the pronuclei. Other individuals that emerge from the agglutinated masses are single, and may remain so. The phenomena are similar in their gross features to the agglutination of diverse types of flagellates, as described by MOEWUS (1933) and others. They resemble also

the agglutination of bacteria under certain conditions; and of blood corpuscles in blood mixtures of diverse types.

But in Paramecium the individuals are so large that they may be observed individually, and opportunity is presented for discovering the exact behavior in agglutination and pairing. The phenomena will therefore be described and illustrated in relation to the usual behavior.

BEHAVIOR IN AGGLUTINATION AND MATING

The behavior system in Paramecium bursaria. The individuals when swimming straightforwardly through the water rotate counterclockwise. That is, if the individual is swimming away from the observer, its upper surface rolls toward the observer's left. At times the animal swims without rotating; this is common in some biotypes, not in others. When not progressing steadily through the water, but feeding or exploring or otherwise under the influence of favorable conditions, the individuals may rotate in the opposite direction; that is, clockwise instead of counterclockwise. Such clockwise rotation is not usually continuous, but occurs only for brief periods. In some biotypes such clockwise rotation is common; in others it occurs very rarely.

But creeping along a surface is in this species much more common than swimming freely through the water. The oral surface is then against the substratum, and the animal moves forward without rotating. It commonly follows a course curving somewhat to the right, in the direction of the right anterior point above described.

To the stimuli commonly met in daily life *Paramecium bursaria* reacts in a manner that is essentially similar to the reactions of *Paramecium caudatum* or *aurelia*, as described in the writer's earlier works (JENNINGS 1906). The "negative" reaction consists in a quick turn to the left (when the oral surface is below); this may or may not be accompanied by a brief stoppage of the forward motion. In case of strong stimulation, the animal may swim some distance backward before giving the characteristic left turn, just as happens in other species.

Of special interest in connection with the mating behavior is the reaction to contact with loose and soft objects, such as fragments of bacterial zoogloea. As in other species, the individual brings itself into close contact with such objects, and may then remain quiet or move gently over the surface of the object. Several or many individuals may react in this way to a small shred of bacterial jelly, so that a dense aggregation is formed, superficially resembling the clumps formed in the mating reaction. But in those due to a small soft object, the animals do not adhere together, but move freely with relation to each other, so that such an aggregation is with

a little practice quickly distinguishable from the mating agglutination, described below.

This contact reaction may occur upon contact with the bodies of other individuals. In such cases two or more individuals may remain for some time in contact, forming a close group. In such groups the bodies move readily upon each other, there being no adhesion of one to the others, as there is in the strong clumping reaction. This contact reaction is closely bound up with the active coordinated reactions of individuals to each other that form one of the two factors in the mating reaction; this is described in the next paragraphs.

BEHAVIOR IN CLUMPING AND PAIRING

Two diverse factors play roles in bringing the individuals together in conjugation. One is an active coordinated reaction of two (or more) individuals, in which they come in contact by some part of their bodies and thereafter move in a coordinated way. This will be called the "active reaction." The other is in typical cases of clumping much more conspicuous; it is an apparently physical adhesion which quickly produces the spectacular clumping mentioned in earlier paragraphs. This will be designated the "agglutination factor." These two will be described and illustrated separately.

1. *Active reaction of two individuals, resulting in coordinated behavior.* When two individuals come in contact by any part of the body, they may or may not react in a coordinated way. In cases in which they do react, the individuals begin to swim in coordinated motion, such as keeps their bodies (or at least their cilia) in contact. The bodies may shift on each other, changing the regions of contact; they may separate for a short distance and again come together; they may swim together for considerable distances, then separate. Or they may remain together, finally uniting in conjugation. Figure 2 shows some of the characteristic phases of this type of behavior. Frequently the individuals are in contact only by the anterior tip of the oral surface, as at A, figure 2. They then swim as a unit, in a spiral course. In other cases the two individuals are placed almost transversely or in the form of an X, the surfaces of the two oral grooves in contact at the region of crossing; the two then swim together in a spiral course (figure 2, C). Not infrequently the cilia of the entire oral surfaces of the two are in contact, so that the two individuals have the relative positions typical of conjugating pairs, save that there is an obvious space between the two bodies, bridged only by cilia. Individuals in this position may swim for a distance together in a spiral course, then partly or entirely separate (figure 2G, a to d), or the relative position may shift. There is great variation as to the regions of contact of the two individuals, though

in the commonest cases it is some portion of the oral surfaces that are in contact.

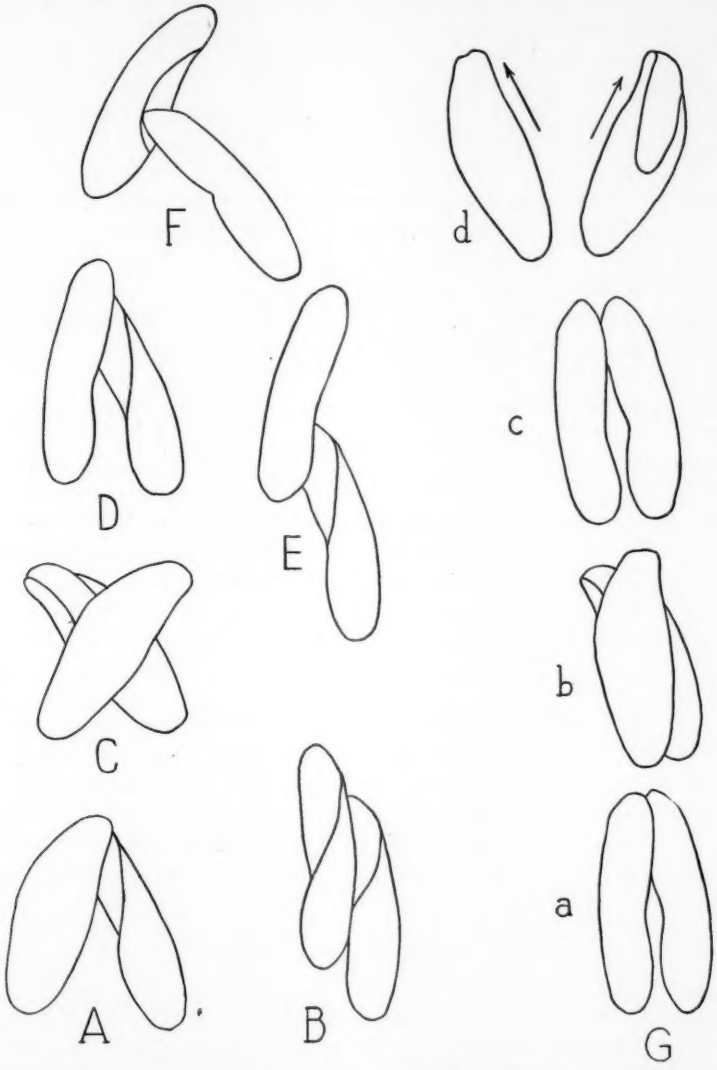

FIGURE 2.—Various positions in the active coordinated movements of two individuals in contact. At G, successive positions are shown (a, b, c, d) in the case of a pair that are in contact by the oral surfaces, swim for some distance in a spiral course, then separate.

In the coordinated spiral swimming of such pairs, it is a singular fact that the rotation is very frequently (but not always) clockwise, though in fully united pairs that are swimming about the rotation is almost always counterclockwise.

A position very commonly observed in such reactions is that shown at F, figure 2. The anterior tip of one individual is inserted into the oral groove at the region of the mouth of the other individual. The two may swim about for some time in these relative positions.

In a type of behavior not infrequently observed, the two bodies are at right angles and in contact at their middle; the two then revolve in such a way that one of the individuals rotates on its long axis, while the other circles about it, as in a spinning top.

In such behavior the relative positions of the two individuals may be varied in an indefinite number of ways; it is frequently shifted during the coordinated motion of the two. It is relatively infrequent that the region of contact for one or the other of the individuals is not a part of the oral surface, as illustrated at B, figure 2. But such cases occur, the motion of the two individuals being coordinated as usual.

Not very infrequently one sees two individuals swimming in a coordinated way, although the distance between them is much greater than can be bridged by the cilia: it may be as great as half the body length or more. Such individuals may swim in a common wide spiral, or one may follow the other, even in an irregular course. In such cases seemingly the water currents must serve as a guide to the movements of the two.

The coordinated behavior just described may occur between individuals of different mating types; it may then lead to closer contact and finally to union by the oral surfaces in typical conjugation. This is frequent when two clones of different type, but only partially mature, are mixed; it may result then in production of a few fully conjugating pairs. But it does not bring about the formation of dense clots, such as occur when mature clones of diverse type are mixed.

It is a remarkable fact that such coordinated reactions and movements are very common in cases in which all the individuals are of the same mating type, so that they do not finally form pairs and conjugate. It may occur when all the individuals are of the same clone, or when two clones belonging to the same (or different) mating types are mixed. It is very common also in immature clones, which do not form conjugating pairs. It must be considered an elemental form of social behavior, which may or may not be connected with the mating process.

Behavior of this general type was early observed and aroused much interest and discussion; see the summary in MAUPAS (1889, pp. 412–414). In the case of Onychodromus, MAUPAS observed that such behavior may

occur between individuals that never fully unite in conjugation (1889, p. 300).

2. *Agglutination Factor.* The agglutination into large clots containing great numbers of individuals, which occurs when mature clones belonging to diverse types are mixed, is due to an additional factor; an actual physical adhesion of the bodies. This will best appear from a description of what occurs when such mixtures are made.

When mature individuals of different mating type (but of the same "group") are mixed together in large numbers, it is to be observed that the individuals are not pulled together or attracted from a distance. They continue to swim about in the same way as in unmixed groups. The swimming is no more rapid or "excited" than when there is no mixture. But the usual movements in varied directions of many individuals in a small quantity of fluid soon brings some of them into accidental contact. An individual of one of the types brushes against one belonging to the other type. Thereupon the two stick together exactly as if their surfaces were covered with some strong adhesive material. There is usually no definite reaction; each of the two individuals tries (as it were) to continue moving as before, but as they stick together, both are suddenly stopped, or one drags the other against the motion of the latter's cilia; or if the two anterior ends happen to lie in the same direction, the two swim forward together.

Any parts of the body that thus come in contact adhere. The two individuals may come in contact by their aboral surfaces, or their rear ends, or in any irregular way; thereupon they adhere, and begin to move in an irregular way resultant upon the divergent action of their free cilia. Figures 3 and 4 show a number of observed cases of such irregular adherence. Often one individual drags another backward or sidewise through the water.

It is the cilia that thus adhere together; at least this is the case at first. Often one may at the beginning see a definite open space between the two bodies, spanned only by the cilia, which are immobilized where in contact. Later the bodies may come into actual contact by their outer surfaces.

Two individuals thus stuck irregularly together flounder about in the crowded drop, and soon come into contact with other individuals of one or the other type. A third individual adheres irregularly to the two, then a fourth, and this continues until large masses are formed, containing twenty to a hundred individuals or more. In these the individuals are in irregular contact, by any parts of the body. In all this it is evident that the coming together of the individuals is the accidental result of their ordinary motions; they adhere when thus accidentally brought together.

Until the individuals come in contact there is no indication of stimulation or of a change in the behavior.

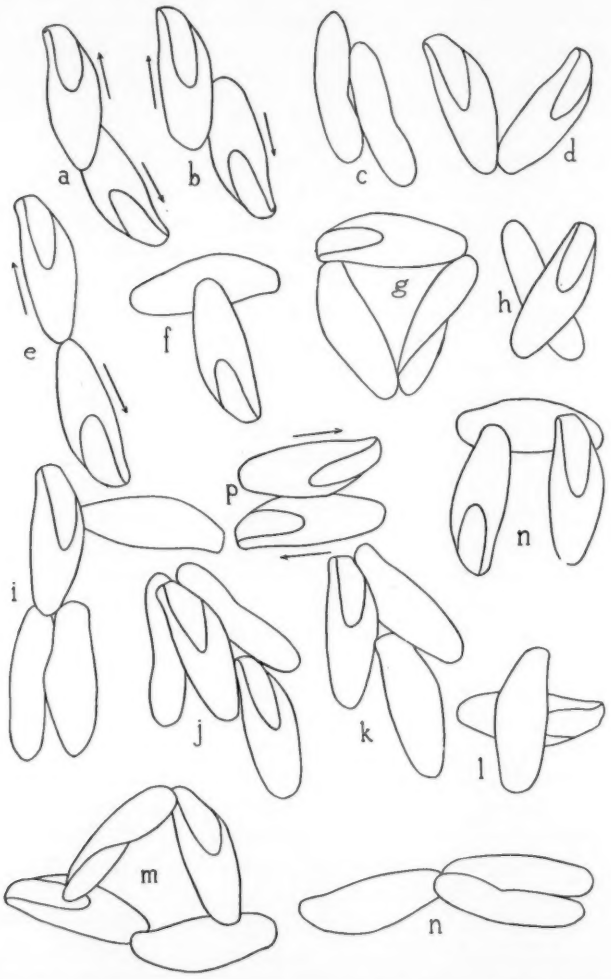

FIGURE 3.—Characteristic small groups formed by the adhesion of individuals in the clumping that precedes conjugation. The arrows present in certain figures show the direction in which the individual's cilia tend to carry it.

By bringing a single individual of one mating type into a collection of many individuals of another type, it may be seen that at times one such

individual becomes the center of a group of five or six or more of the other type, each of the latter adhering to it by some small part of the body surface. In all cases the adhesion is between two individuals belonging to different types; members of the same type do not adhere together. Thus the large clumps contain approximately equal numbers of individuals of the two types. In the mixtures containing many individuals of both types, small groups are at first formed with all sorts of irregular attachments. Some sketches of small groups are shown in figure 3.

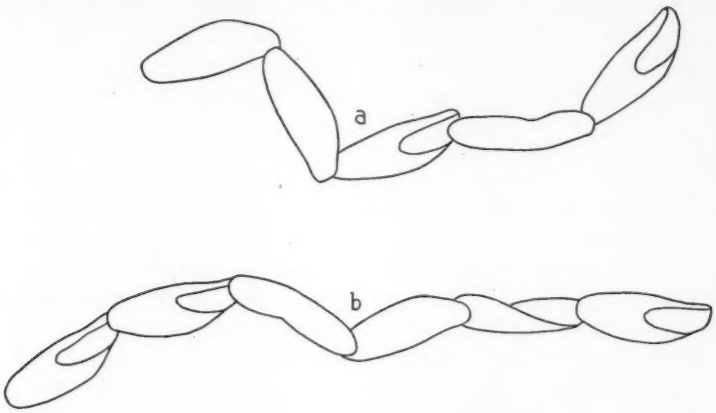

FIGURE 4.—Chains of individuals adhering together at their tips, in a late stage of the agglutination that leads to conjugation.

Within three or four minutes large masses of irregularly adhering individuals are formed. They commonly remain united for about half an hour, then begin gradually to break up into smaller masses. Yet (unless evening is coming on) masses of considerable size may thus remain united for two hours or more. As the masses break up many longitudinal chains are found to have been formed, the individuals united end to end (figure 4), individuals of the two types alternating. In such chains most (but not always all) of the individuals are oriented in the same direction, the anterior end of one individual overlapping slightly the posterior end of the one in front of it. Usually if not always it is now the anterior part of the oral groove that adheres to the side of the posterior part of the individual in front; it may adhere to either the oral or aboral surface or to either lateral surface of this posterior part. At times, such chains have one or more individuals reversed, so that these latter are dragged about with posterior end forward. In other cases two or three individuals are attached irregularly to the sides of the chain. Such chains of 5 to 20 or more in-

dividuals swim about in a snake-like manner, most or all of the individuals being oriented with anterior ends in the same direction, so that their motion is coordinated. At times the chains are zigzag in form.

In the clots above described the individuals adhere firmly together by whatever parts of the bodies are in contact. They do not move freely with relation to each other, as occurs in the groups described earlier, that result from the actively coordinated contact reactions. In all the clumping process the coordinated contact reaction plays little or no part, the main or exclusive factor being the rigid physical adhesion of individuals of diverse type that are in contact.

In immature or partly mature clones however this agglutination factor may be nearly or quite lacking; there is no physical adhesion of individuals that come in contact. In such cases scattered pairs may be formed seemingly as a consequence of the active coordinated contact reaction only (as before mentioned). In partially mature clones the physical adhesion becomes slightly developed, so that cases are seen in which two individuals react in the coordinated way earlier described, but if they try to swim apart they are found to adhere slightly. The physical adhesion factor increases as the clone grows older, so that at certain periods mating appears due to a nearly equal mixture of the coordinated contact reactions and the physical adhesion. When the clone becomes fully mature, in typical cases the clumping and mating appear due almost exclusively to the physical adhesion.

Yet even in the tightly adhering groups formed by agglutination the individuals may shift to some extent, moving slowly and with seeming difficulty. In this way the relative positions become slowly changed and some of the individuals come in contact by their oral surfaces; in time they come into the typical mating position. This is a slow process, requiring usually an hour or more, so that the exact method by which the final position is attained is difficult to detect. At first the pairs are united only by the oral cilia, so that there may be a space between the bodies. Later they come closer, until they appear to be fused; they then swim about as a single body, rotating counterclockwise.

Not all the individuals that are incorporated in the agglutinated masses finally become united in pairs. After some hours the masses are quite disintegrated, and it is found that many single individuals, as well as many pairs have separated from them. Nothing now remains but pairs and single individuals. As described below, toward evening the tendency to adhere weakens and then fades away completely, so that only those individuals that have become fused to pairs remain in union.

As set forth in my preliminary communication (JENNINGS 1938), it can readily be demonstrated that the two members of any pair belong to the

two diverse types that have been mixed. Before mixture one of the types is induced to multiply rapidly, so that the algal cells which each individual contains are few, the individuals are therefore white, like *Paramecium aurelia*. The other type is allowed to remain of the typical deep green. When the two are mixed, the pairs formed always consist of one green individual, one white one.

The clotting and pairing take place in the commoner Groups I and II most strongly at about midday and for three or four hours after; but in Group III they may occur at any hour of the day or night. In the former two groups the tendency to clot when mixtures are made begins at about 8 a.m., becomes more marked as the day progresses, then at 4.30 or 5.00 p.m. begins to weaken; it may have completely disappeared by 6.30. In mixtures made in the evening from 7.00 on there is in these groups no clumping and pairing. A detailed investigation of the time relations and the relations to light and darkness is in progress.

In mixtures containing very large numbers of the two mating types, there is usually during the first day a strong clumping, and many pairs are formed. In some cases however there is hardly any clumping during the first day; this is probably the result of marked differences in the fluids in which the two sets were living. In such cases the first marked reaction comes the second day. In the evening the clotted masses have separated, many individuals being left single. The next day there is a renewed agglutination, with formation of additional pairs. On the third day there is again agglutination and pairing among the left-overs of the previous day. In some cases the reaction by this time has gone as far as it will, so that the next day there is no more clotting. But if the numbers are large, agglutination may be repeated on later days. In a certain case large numbers of two types of Group I were mixed in a Columbia dish on June 7. Agglutination and later dispersal occurred daily until June 17, and a few cases of adhesion of individuals occurred for two or three days longer.

The account of agglutination and the mating behavior just given is typical for mature stocks, including most clones derived from individuals collected under natural conditions. There are however some such clones that react in a much less pronounced manner. In some clones but a few individuals out of many show the tendency to agglutinate and pair. In these cases only small clots are formed, of two to five or six individuals; and later only a few pairs are found. In the strength of the tendency to agglutinate and pair many different grades are found among different clones collected in nature, from an entire absence to the typical strong tendency that results in the dense aggregations that are typical. In some clones, pairing occurs seemingly quite without agglutination, simply through the active contact reaction described in earlier paragraphs.

Some of the individuals collected in nature yield clones that for long periods do not agglutinate or pair, but later these mating reactions begin and progressively become stronger. Thus, five clones collected in July 1937, although frequently tested, gave no clotting or mating reactions until March 1938, when scattered unions occurred on mixture with typical clones. The clotting and pairing gradually increased until it became possible to assign the five clones to certain mating types of Group II. Other clones have been for months in the laboratory, but have as yet shown no sign of the clotting or mating reactions, when mixed with other clones, although they may, whether mixed with others or not, show the active coordinated contact reactions earlier described. It seems probable that such clones are in most or all cases immature (see the account of immaturity in a later section).

LENGTH OF TIME OF UNION

After the individuals have become united in pairs, union continues for from 24 to 48 hours. The time of union and separation was observed for 12 pairs belonging to Group I. The total time during which the two individuals remained united was as follows: one pair remained in union for 26 hours; six pairs for 28 to 31 hours; five pairs for 44 to 47 hours. These periods are in good agreement with the observations of MAUPAS (1889, p. 229), who found that at 20 degrees Centigrade the period of union was 36 hours. The fact that the union continues for so long a time is convenient for many purposes in the study of conjugation, since if a test mixture of types is inspected once in 24 hours all pairs formed may be observed.

CONDITIONS REQUIRED FOR CLOTTING AND CONJUGATION

Under usual conditions most clones derived from individuals collected in nature clot and pair in the typical manner described above, when mixed with others of a different mating type (but belonging to the same group). Certain conditions however may interfere with or prevent these mating reactions; some of these are intrinsic, others extrinsic. They may be classified as follows:

1. *Immaturity.*—For a varying period of considerable length after conjugation, the descendants of ex-conjugants neither agglutinate nor pair when mixed with other types. There is a period of immaturity during which the mating reaction does not occur. The tendency to react, when it first appears, is extremely weak and sporadic; so that in a mixture with a typical clone of a different type only a few scattered individuals react and pair. The first pairs are in some cases seemingly formed without any participation of the agglutinating factor, through the active contact reaction only. In other cases single individuals are seen to adhere in the agglutina-

tion reaction to single individuals of the other type. As the days or weeks or months pass a larger proportion of the individuals of the clone are seen to react, so that several or many pairs may be found in a mixture. Finally the typical agglutination and pairing phenomena are observed. Sometimes it is months from the appearance of isolated pairs till the time when all react strongly. During this period of partial immaturity the occurrence of agglutination and pairing depends in a high degree on the conditions; unless the most favorable conditions are supplied no pairing occurs.

The period of complete immaturity, when no mating reaction at all occurs, varies from two or three weeks up to many months. The shortest period thus far observed from conjugation to the beginning of the mating reaction was 12 days, in the case of a clone descended from a mating of the Types A and C of Group I; at this time but few individuals reacted, and these but faintly. In 25 other clones descended from diverse ex-conjugants of the same mating there was no mating reaction at this time; their reactions began later. Many clones descended from conjugations that occurred in Group II in April and May, 1938, are still without mating reactions in November of that year. The different clones descended from the ex-conjugants of the same epidemic of conjugation, between the same two parental clones, show very great diversities in the time when the mating reactions begin, and in the time when the reaction reaches its full strength.

The usual long period of immaturity, complete or partial, is inconvenient for genetic experimentation. Young clones produced by a given mating must be cultivated in isolation for long periods before the reaction type to which they belong can be determined.

On the other hand the definiteness of the reactions when mature clones are mixed presents a remarkably favorable opportunity for the study of the different periods of the life history, so much insisted on by MAUPAS (1889), CALKINS (1935) and others: youth, maturity, age. Detailed studies on these matters are in progress and will be presented later.

2. *Nutritive condition.*—Clotting and mating do not occur when individuals to be mixed are taken from a rich nutrient medium in which they are plump and rapidly dividing; nor, usually, when the mixture is made in such a medium. In general, plumpness of body tends to prevent the mating reaction. Starvation is not necessary; clotting and mating occur readily in well-fed well-grown individuals, provided their plumpness is not excessive.

Mature strong clones show little dependence on nutritive condition; they clot and mate whenever mixed, unless excessively over-fed and plump. But immature or refractory clones are extremely sensitive to the nutrient condition. The most favorable conditions for the mating reaction in such cases are found when the nutritive conditions are declining from

a rich condition to a moderate or poor one. Such clones do not react either when plump, or when kept for some time without food. In practice, to induce reactions in immature or refractory clones, the following procedure is helpful. The individuals from the clone to be tested are mixed with mature individuals of known type in two or three drops of pure algal nutrient medium described in earlier paragraphs. This induces multiplication, but there is no pairing for several days. After three, four or five days however the nutrient medium is becoming exhausted, the individuals are declining in size, and now mating may occur. By this means mating may be often induced in clones that otherwise do not show it.

But very great differences in the effects of nutritive conditions on agglutination and mating are seen among the different young clones derived from ex-conjugants of a given mating. Some such clones have been observed in which addition of the strong nutrient medium to the mixture brought about the formation of pairs within 24 hours, while the animals were still plump from the increased food.

3. *Time of day.*—As before set forth, the mating reactions occur only at certain periods of the day; usually not at all in the evening after six or seven o'clock, nor in the morning from five to eight or nine o'clock. The most favorable time is for most types from about twelve noon to four p.m. The most favorable period for reaction differs in different groups and clones. A detailed study of this matter will be presented later.

4. *Shock of change from one fluid to another.*—It is of course easy to prevent the mating reactions by mixing the animals in fluids that are irritating or injurious. The common accidental differences between cultures of diverse clones often greatly delay the reactions when clones of different types are mixed. In such cases, when the two clones are mixed, clotting and mating may not occur until the next day, coming on in the favorable period of that day; it may then be as marked as when it occurs at once. In many cases however even marked differences of culture medium produce no interference with the reaction; the tendency to clot is so strong that it at once overcomes such diversities.

DIVERSE MATING TYPES; AND DIVERSE GROUPS OF TYPES

As mentioned earlier, the members of a single clone (all derived by fission from a single individual) do not clot nor mate together, under ordinary conditions. The single clone is self-sterile, in the same sense as certain plants are self-sterile (East 1929), and as the animal Ciona is self-sterile (Morgan 1938). The self-sterility is not complete, and as in other organisms, the usual term "self-sterility" does not accurately characterize the phenomena, since there is likewise cross-sterility of certain diverse clones.

If members of a single clone are mixed with members of another clone,

clotting and conjugation may occur, in the manner described above. (For brevity, the complex of phenomena will be called the mating reaction.) But a given clone does not react with members of all other clones; with some other clones reaction is lacking. This selective reaction with certain clones and failure to react with others is found to include two diverse sets of phenomena; those leading on the one hand to the distinction of diverse mating types; and those leading on the other hand to the distinction of different groups of types. It will be convenient first to present the phenomena in the case of a single group.

If individuals are collected from a certain pond and by the isolated culture of single individuals a large number of clones are obtained, the following phenomena are observed. Members of the same clone do not react together. Members of a given clone react with those of certain other clones, while with still others they do not react. Two clones whose members do not react together may be said to belong to the same mating type, since their members have in this respect the same relation to each other as have the members of a single clone. On the other hand two clones whose members react together when mixed belong to diverse mating types.

By making all possible mixtures among the different clones, it is found that a number of diverse mating types exist. Clones of type A react with clones of type B, of type C, of type D, and so on. Similarly, clones of type B react not only with A, but also with C, D, and so on.

Extensive study shows that the number of diverse mating types is limited. In collections from a pond in the neighborhood of Baltimore, clones were found that belonged to five diverse mating types, which were designated as follows (from the designations of typical clones of each type): Fb, Fd, Fl, Fo and S. The relation between these types is this: members of any single type, as Fb, will not react with members of that same type, but will react with members of all the other types (with Fd, Fl, Fo, and S). Each type includes many different clones that do not react together. These five diverse types are members of what was called in my preliminary paper (1938) the Maryland Group, or Group II; the same designation is here used.

Since that paper was published, three more mating types belonging to this Group II have been discovered; these were designated from the typical clones first found as Gr6 and Gr14 (from collections made in North Carolina) and McD3, from a collection made near the site of the McDonough School near Baltimore. Thus this Group II ("Maryland Group") is now known to contain eight diverse types, of such relations that the individuals of any one type will react with those of any of the other seven types, but not with members of its own type. Tests of a very great number of clones of this group, collected in nature or bred in the laboratory have

shown that all belong to one or another of these 8 mating types, so that there is some reason to believe that Group II consists of just 8 mating types and that no others will be found belonging to this group.

But the members of certain other clones do not react with any of the eight types of Group II, yet do react with certain clones. These clones that react together constitute another group, with a number of different mating types, members of which react together, but not with any of the types (or clones) belonging to Group II. In my preliminary papers (1938) I described such a group, known as Group I, or the "Virginia Group." This group contained four diverse mating types, designated (from typical clones) as l, m, 67a1, and 1b1. When two types of this group are mated, they yield among their offspring the above four types and no more. Thus there is ground for believing that there are but these four types in Group I.

Since my preliminary papers were published another group, Group III, has been found, originally in material sent me by DR. COLEEN FOWLER, from collections made between Greensboro and Pineville, North Carolina. This Group III is known to contain (like Group I) four diverse types which were originally designated (from clones representing them) as Gr1, Gr2, Gr11, and Pi13. Any type of this group will react with any other type of the same group, but will not react with any of the types belonging to Group I or Group II. This Group III has thus far been studied less than the other two groups. Possibly other types belonging to it will be found, though it seems not improbable that, like Group I, it consists of just four types.

Any clone that is found to react (by agglutination and mating) with any type belonging to a particular group (as Group I) is invariably found to react with all the other types belonging to that Group, *except one*—this one constituting the type to which the clone under examination belongs. Furthermore, after a clone has been found to react with members of a given group (as Group I), it is found that it does not react with any of the clones of the other two groups.

After the three diverse groups, each with several types, had been found in the eastern part of the United States, the author anticipated that collections made in distant regions would show still other groups. But collections sent me from California (from a pond on the grounds of Stanford University) contained only individuals belonging to the commonest type E (Fd) of the large Group II, which is abundant in Maryland and other states of the Atlantic region. Again the four types of Group III were originally found in North Carolina, and for a long time were not known to occur in other regions. But in the summer of 1938 collections from Provincetown, at the tip of Cape Cod, Massachusetts, were found to consist entirely of one of the types (P) of Group III. Some of the groups

are therefore certainly widely distributed. It is possible however that other groups in addition to the three now known will be discovered on fuller investigation.

So far as at present known, therefore, the species *Paramecium bursaria* consists of three groups, the members of any one of which do not mate with members of the other two. Groups I and III contain each four diverse mating types, while Group II contains eight mating types, so that there are in all sixteen mating types. The constitution of the species, so far as now known, may be represented as in the adjoining figure 5.

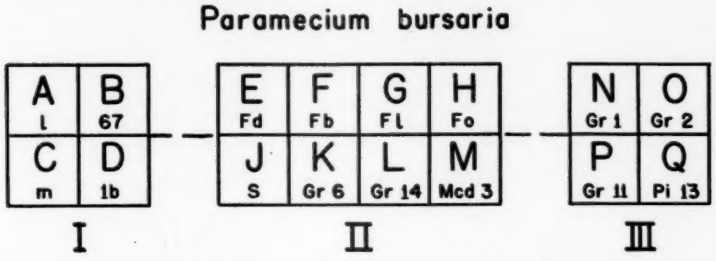

FIGURE 5.—Constitution of the species *Paramecium bursaria*. Three groups, I, II, III, that do not intercross; sixteen mating types A to Q; four in Group I, eight in Group II, four in Group III. The small letters or figures in the lower half of the squares are the designations employed in the investigation, from those of the clones that first exemplified each type.

The interactions of the 16 diverse types belonging to the three groups are shown in the table of figure 6. In this table the plus sign indicates that agglutination and conjugation occur, with formation of fertile ex-conjugants; the minus sign that they do not.

It will be convenient to designate (as in figure 5) the 16 different mating types of the three groups by the first 16 capital letters of the alphabet (excluding the letter I as ambiguous); that is, by the letters A to Q. The four types A to D constitute Group I; the eight types E to M, Group II; the four types N to Q form Group III. In figure 5 there are given also for convenience the more cumbersome designations that have been employed in the practice of the investigation; these are the designations of the clones that were first found to exemplify each type (as l, 67, Fd, Fb, etc.).

OCCURRENCE AND DISTRIBUTION OF THE TYPES AND GROUPS

When collections are brought into the laboratory, the following is the procedure in determining the groups and types represented. A considerable number of single individuals are isolated from the collections and cultivated in isolation till abundant clones are obtained from each. In the laboratory are kept cultures of the 16 known diverse reaction types—the

four of Group I, the eight of Group II, the four of Group III; these may be known as testers.

The necessary tests are carried out on slides each of which carries two depressions. For the preliminary test three slides, with their six depressions, are required. From the clone to be tested a large number of individuals (50 to 100 or more) are placed in each of the six depressions.

Paramecium bursaria

		I				II								III			
		A	B	C	D	E	F	G	H	J	K	L	M	N	O	P	Q
I	A	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	B	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	C	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−
	D	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
II	E	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	−
	F	−	−	−	−	+	−	+	+	+	+	+	+	−	−	−	−
	G	−	−	−	−	+	+	−	+	+	+	+	+	−	−	−	−
	H	−	−	−	−	+	+	+	−	+	+	+	+	−	−	−	−
	J	−	−	−	−	+	+	+	+	−	+	+	+	−	−	−	−
	K	−	−	−	−	+	+	+	+	+	−	+	+	−	−	−	−
	L	−	−	−	−	+	+	+	+	+	+	−	+	−	−	−	−
	M	−	−	−	−	+	+	+	+	+	+	+	−	−	−	−	−
III	N	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
	O	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+
	P	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+
	Q	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−

FIGURE 6.—Table of the interactions of the 16 mating types A to Q of the three groups of *Paramecium bursaria*. The plus signs indicate that clumping and conjugation occur between the two types indicated; the minus signs that they do not.

Then to the first two depressions are added numerous individuals of two of the types of Group I (one of the types to each depression); to the third and fourth depressions are added individuals of two of the types of Group II; to the fifth and sixth depressions, two of the types of Group III. Thus each of the six depressions contains a mixture of individuals of the clone to be tested with individuals of one of the types.

The six mixtures are then examined under the binocular. The clone to be tested will as a rule be found to clot and pair with one or both of the types of one of the groups; it is thereby shown to belong to that group. If the new clone belongs to a certain group (as Group I), it is certain to

react (if in proper condition) with at least one of the two tester clones of that group. For every clone belonging to a certain group reacts with all the types of that group except its own type. In this way it is discovered to which group the clone belongs. Suppose that it is thus found to belong to Group II. It is next necessary to test it, in the same manner, with each of the six remaining types of that group. It is invariably found that the new clone reacts with seven of the eight types of Group II, but not with the eighth. The one with which it does not react is the type to which it belongs. In a similar way, if it is found to belong to Group I or Group III, the type to which it belongs is discovered by testing with the remaining members of that group. The results of the tests are in typical cases sharply defined; either there is the spectacular clotting and pair formation described in earlier pages, or there is no reaction. The type to which the clone belongs is absolutely clear. In rare cases however, some of the clones are refractory; they do not form pairs with any of the 16 diverse types. If such clones are retested at intervals of weeks or months, some of them develop the capability of reacting, and the type and group to which they belong may be determined.

By the use of these methods the occurrence and distribution of the different types and groups were determined as shown in the following list. The collections were partly made by the author; for other collections from various parts of the United States I am greatly indebted to the following: Dr. Coleen Fowler, Dr. Zdenka Hurianek, Dr. Elizabeth S. Kirkwood, Miss Amicia Melland, Dr. Kathryn Stein, Mr. N. Finkelstein, Dr. John Frisch, Dr. A. C. Giese, Dr. N. Higginbotham, Dr. Richard Kimball, Dr. Austin Phelps, Dr. T. M. Sonneborn, Mr. M. A. Wessel.

Group I. Four Types, A, B, C, D (or l, 67, m and 1b)

Types A and C were first collected from a pond at Alexandria, Virginia, as clones l and m, by Dr. T. M. Sonneborn. Later by the crossing of these two were produced types B and D (as clones 67 and 1b). Still later all four were collected from various natural habitats, as follows:

Type A. Two clones (l) from ponds at Alexandria, Va.; five from pools near Hebbville, Md.; three from a pond in Loudon Park Cemetery, Baltimore. Produced many times by crossing.

Type B. First produced (as clone 67) from a cross of Type A and Type C. One clone found in Loudon Park Cemetery, Baltimore; one from a pool near Texas, Md.; one from Jones' Falls at Dickeyville, Md.; one from pool at Hebbville, Md.

Type C. One clone (m) from a pond at Alexandria, Va.; one from

Staunton, Va.; two from pond at Loudon Park Cemetery, Baltimore. Produced many times by crossing other types.

Type D. First observed as a clone (1b) from a cross of types A and C. Seven clones collected from pool in Loudon Park Cemetery, Baltimore; six clones from pools near Hebbville, Md.; six from pool near Westminster, Md.

Thus the four types of Group I have been collected as 37 clones, from regions thus far limited to Maryland and Virginia. The 37 collected clones were distributed among the four types as follows: 10A, 4B, 4C, 19D.

The distribution of types in collections from particular localities is illustrated in the following:

Pool at Westminster, Md., six clones, all Type D.

Pool at Hebbville, Md., April 2, 1938; 12 clones: 5A, 1B, 6D. A later collection from the same pools gave clones that all belonged to Group II.

Pool in Loudon Park Cemetery, Baltimore; 13 clones: 3A, 1B, 2C, 7D.

Pool at Staunton, Va.; 23 clones, 1C; the other 22 belonging to Group II.

It appears probable that farther collections will show a much wider distribution of Group I.

Group II. Eight Types, E to M

Type E (or Fd). 45 clones from pools and ponds in the region about Baltimore; six from Hope Valley, Rhode Island; one from Compton, Rhode Island; four from Staunton, Virginia; 76 from a pool on the campus of Stanford University, California. In all, 132 clones collected from natural habitats.

Type F (or Fb). 14 clones from ponds and pools in the region of Baltimore; one from a stream in the southern part of North Carolina; two from Hope Valley, Rhode Island; 12 from ponds at East Aurora, New York; three from pond at New Haven, Conn. In all 32 clones.

Type G (or Fl). Five clones from about Baltimore; four from Staunton, Virginia; one from near Greensboro, North Carolina; one from Hope Valley, Rhode Island; two from Miller's Pond, Montville, Connecticut; 12 from East Aurora, New York. In all, 25.

Type H (or Fo). Seven clones from about Baltimore; three from Staunton, Virginia; three from pool in the plant house of Mt. Holyoke College, South Hadley, Mass.; three from Miller's Pond, Montville, Connecticut; seven from a pond at New Haven, Conn. In all, 23.

Type J (or S). 15 clones from about Baltimore; 11 from Staunton, Virginia. In all, 26.

Type K (or Gr6). 11 clones from about Baltimore; three from south of Greensboro, North Carolina; three from Miller's Pond, Montville, Rhode Island; one from the Cedar Swamp, Woods Hole, Mass. In all, 18.

Type L (or Gr14). Eight clones from about Baltimore; one from south of Greensboro, North Carolina; 12 from the Greenhouse at Columbia University, New York; one from Hope Valley, Rhode Island; one from Miller's Pond, Montville, Conn.; seven from New Haven, Conn. In all, 30.

Type M (or McD3). Two clones from about Baltimore; two from Hope Valley, Rhode Island; one from Compton, Rhode Island; four from New Haven, Conn. In all, 9.

As the above list shows, the eight types of Group II are very widely distributed, ranging from Cape Cod, Mass., in the east to California in the west, from East Aurora, New York, in the north to the southern part of North Carolina in the south. In the collections thus far made some of the types occur much more frequently than others. Of Type E (Fd) 132 clones from many localities have been found; of Type M (McD3) but nine. In a collection from a quarry hole at Texas, Maryland (near Baltimore), all the eight types were found to be present. Possibly fuller investigation would show that it is not uncommon for this to occur.

Group III. Four Types, N to Q

Type N (or Gr1). Three clones from a stream 50 miles south of Greensboro, North Carolina; four from a stream not far from Pineville, North Carolina.

Type O (or Gr2). Two clones from 50 miles south of Greensboro; four clones near Pineville—both in North Carolina.

Type P (or Gr11). One clone from 50 miles south of Greensboro, North Carolina; 14 from Provincetown, Cape Cod, Massachusetts.

Type Q (or Pi13). Three clones from near Pineville, North Carolina.

INDEPENDENCE OF THE THREE GROUPS; DISTINGUISHING CHARACTERISTICS

The three groups remain independent, not intercrossing, so far as my observations and experiments go. Clones that react strongly invariably clot and form pairs with members of one of the three groups, not with those from the others. If such strong clones belonging to two diverse groups are mixed, there is no clotting nor pair formation.

When, however, members of immature or refractory clones are tested with members of different groups, in very rare cases single pairs are formed in mixtures with members of more than one group; that is, isolated pairs are rarely formed with members of a group to which the immature or refractory clone does not belong. On repetition of the mixtures, no additional pairs are obtained; the pair found remains a unique example. Such pairs have been observed in but half a dozen out of hundreds of mixtures made. In two or three cases attempts were made to cultivate the ex-conjugants

of such pairs, but without success. As set forth later, self-fertilization of clones occurs in very rare cases; it remains possible therefore that these isolated pairs were not crosses, but the result of self-fertilization in one of the two clones mixed. The matter requires thorough investigation, which will be difficult, owing to the extreme rarity of the occurrence of such pairs.

Are the three groups distinguishable by other characters than their mating reactions? As has already been mentioned, Group III differs from the other two groups in the fact that in Group III the mating reactions may occur at any time of day or night, while in Groups I and II they occur mainly or exclusively in the day time, particularly in the afternoon. There are indications also that the cultural conditions required in Group III differ slightly from those in Groups I and II. To determine whether there are structural and morphological differences between the groups will require more detailed studies than have yet been made. Such studies are in progress, the cytological comparisons being undertaken by DR. T. T. CHEN. All the groups, however, have the general form, structure and size that is characteristic of *Paramecium bursaria;* all have the type of micronucleus known for this species, and all contain the characteristic green algal cells.

When particular clones belonging to diverse groups are compared, often there are marked differences in size, form, or other respects, between them. But such differences are usually merely characteristic for the particular clones examined, and are not general for the two groups. This matter is taken up in connection with the question of diversity of the mating types, in the next section.

CLONAL DIVERSITY IN RELATION TO DIVERSITY OF MATING TYPES

Different clones in any of the groups differ greatly in size and form of the individuals as well as in physiological characteristics. The size, form, and physiological peculiarities of a clone remain characteristic of it for long periods; they are transmitted in multiplication by fission. Such characteristic differences in size and shape are shown in the outlines of typical individuals of certain clones, all shown to the same scale in figure 7. As the figure shows, clones very diverse in these ways may belong to the same mating type or to diverse mating types (the type as well as the designation of the clone is indicated in the description of figure 7). Observe the marked differences in size and form of numbers 1 and 2 of figure 7, both belonging to type A; of 11 and 12, belonging to type E, and in other cases. The situation observed is that which would exist if mating type, size, shape, and other peculiarities depend on diverse factors distributed independently. If there are any characteristics that go unvaryingly with

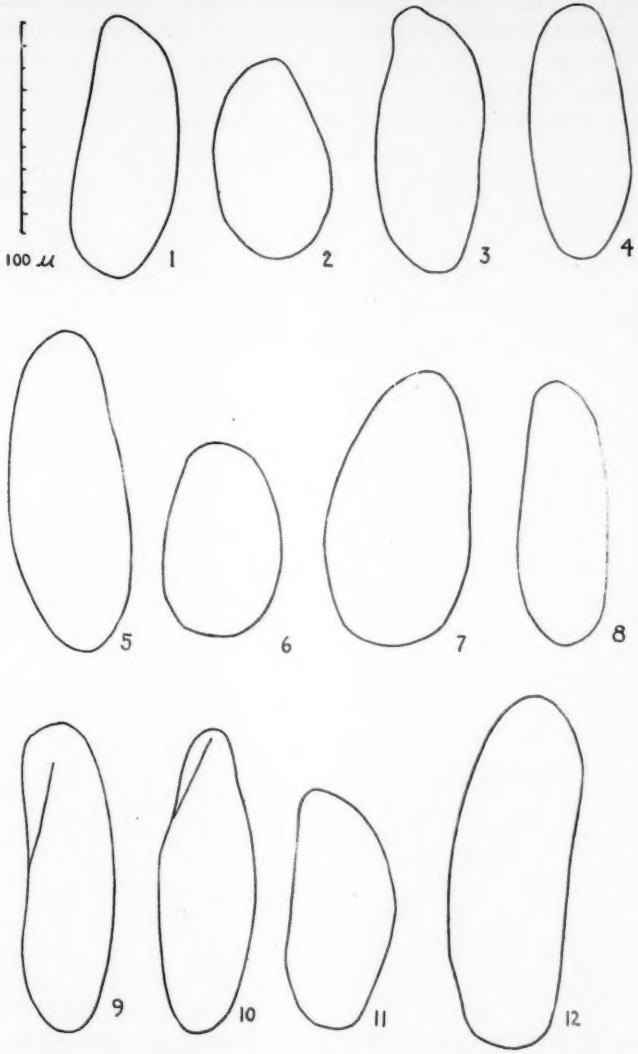

FIGURE 7.—Outlines of typical adult individuals of diverse biotypes of *Paramecium bursaria*, all under approximately the same conditions. Camera figures, all drawn to the same scale.

1, Type A, clone Lo2. 2, Type A, clone l. 3, Type C, clone m. 4 and 5, Type B, clone 67. 6 and 7, Type D, clone 1b. 8, Type D, clone Lo1. 9 and 10, Type M, clone McD3. 11, Type E, clone Fd. 12, Type E, clones from Stanford University, California.

a particular mating type or a particular group, these are so slightly marked as compared with great clonal differences that are independent of type and group, that they have not yet been detected. A great variety of combinations of morphological and physiological characters may be found in any mating type or group.

PERMANENCE OF THE MATING TYPES

Any single clone kept in mass culture in the laboratory and multiplying vegetatively, appears to retain indefinitely its characteristic mating type. Clones l and m, of types A and C, respectively, were brought into the laboratory in April 1937; they are now, in October 1938, of the same types A and C. Many other clones have been in mass culture in the laboratory for more than a year; all still react as the same mating type as at the beginning.

In *Paramecium aurelia*, according to SONNEBORN (1937) and KIMBALL (1937), there is commonly a change of type in a part of the individuals at endomixis, so that a clone that originally belongs to but one of the two mating types has after endomixis both mating types, which may then conjugate together. As endomixis occurs in *Paramecium aurelia* every two to four weeks, there are in that species frequent changes of mating type; a mass culture of a clone does not remain pure for a certain type. In *Paramecium bursaria* endomixis is seemingly infrequent. ERDMANN (1927), who examined into this, did not find endomixis to occur at all in isolation cultures, but did find all stages of the process in mass cultures. The clones l and m, above mentioned, have been kept in mass cultures in the laboratory for 18 months, so that if endomixis is a regular occurrence, it seems probable that the process must have been undergone several times; yet the original mating type is retained.

The retention of the mating type appears the more remarkable in view of the occasional occurrence of self-fertilization in a clone, as set forth in the next paragraphs.

Self-Fertilization. Any clone (derived by fission from a single individual) belongs, under usual conditions, to a definite mating type; it forms clumps and conjugating pairs only with members of other types of the same group.

But in very rare cases a pair, or more than one, is found in a mass culture of a single clone; or in a mixture of two clones that by all other tests belong to the same mating type. Conjugation has occurred between members of the same clone, or members of two clones that appear of the same type. This occurs so rarely that one may have clones under frequent examination for months without observing a single pair. But as months pass the number of such observations accumulates. In view of the fact that in *Paramecium aurelia* differentiation into two types is known to occur at endo-

mixis, it would seem probable that this production of pairs within a single clone or single mating type is due to a differentiation, by endomixis or in some other way, into two or more diverse mating types, which then conjugate. The genetics of self-fertilization in *Paramecium bursaria* will be treated separately, in a paper to appear soon; there will be discussed also in the light of facts brought out, the seemingly permanent retention of the original mating type by clones kept for long periods.

Clones that do not conjugate. As before mentioned, when large numbers of individuals are collected from natural habitats and clones are obtained from them, some clones are found that do not react with any of the 16 mating types of the three groups. In some cases these are presumably immature clones; the mating reactions develop later, as before set forth. But some clones have never developed mating reactions. The clone Gr4 has been in the laboratory in mass culture since February 25, 1938; that is for more than eight months, yet has never reacted in any of the frequent tests given it. During that period many clones have been produced by conjugation, and have developed to maturity, so that they give strongly marked mating reactions. Other clones that have never reacted have been for long periods in the laboratory.

Such refractory clones may possibly be clones in which the period of immaturity is very long. Great genetic differences in the length of the period of immaturity are known to occur. Or such clones may be genetically defective, lacking entirely the tendency to conjugate. Or, finally, they may belong to other groups of mating types, in addition to the three above described. If no other members of such additional groups are present in the laboratory, there would be no opportunity for such clones to display the mating reactions.

GENETIC RELATIONS

In my preliminary paper (1938) certain general genetic relations were set forth. In Group I, by the conjugation of types A and C the two additional types B and D were produced; that is, conjugation of A and C produced all the four types of Group I. In descendants of ex-conjugants from mating of the members of two clones of diverse type, as A and C, usually but not always the descendants of the two ex-conjugants of a given pair are of the same mating type. But in some cases they are of diverse mating type. Further, in rare cases the two clones descended from the two individuals produced by the first division of an ex-conjugant are of diverse mating types, so that a segregation of the sex types may occur at the first division after conjugation, as in *Paramecium aurelia* (SONNEBORN 1937).

An extensive study of the genetic results of crossing and self-fertilization of clones in the various groups is in progress and will be reported in later contributions.

Historical

The fact that in the Vorticellidae the two members of a conjugating pair differ greatly has, of course, long been known; the early history of this matter is given by MAUPAS (1889, pp. 380–385). But with the free swimming ciliates the case is far otherwise.

In his great paper of 1889 MAUPAS records certain important observations and experiments which may now be interpreted as indicating the existence of diverse mating types in certain of the free ciliate infusoria. In four species, *Leucophrys patula*, *Onychodromus grandis*, *Stylonychia pustulata* and *Loxophyllum fasciola*, MAUPAS found that no conjugation occurs in a culture in which all the individuals are derived from the vegetative multiplication of a single parent (that is to say in a single clone). but commonly does occur if two such clones are mixed. This he says is based upon "several hundred" experiments of this type on the four species mentioned (see MAUPAS' general summary on this point, 1889, pp. 410–411). MAUPAS naturally concluded that the essential point here is the close relationship of the individuals that will not conjugate together. While this idea had a foundation of truth, it is now clear of course that it is not an accurate or adequate expression of the situation. On the one hand, as shown in the present paper, clones of entirely different descent, and coming from regions thousands of miles apart (California and Connecticut), may belong to the same mating type, so that they do not conjugate together. On the other hand, two clones so closely related as to have been derived from the same ex-conjugant may in some cases belong to diverse mating types, so that they readily conjugate and produce viable descendants.

The observations and conclusion of MAUPAS, just cited, appear not to have been taken up and developed for the ciliates until the appearance of the recent paper of SONNEBORN (1937). The present writer examined earlier into the matter, selecting for intensive study *Paramecium aurelia*. But in that species, as it turns out, conditions are exceptional and very diverse from those described by MAUPAS. In *Paramecium aurelia* conjugation frequently occurs between members of the same clone; it may occur even in cases in which the two individuals that conjugate are but four or five vegetative generations from the same ancestor; and such pairs produce viable offspring which again conjugate among themselves (JENNINGS 1910). This led to a turning aside from the study of the effects of relationship on conjugation.

But SONNEBORN in his studies of the results of endomixis, in this laboratory, found that in *Paramecium aurelia* endomixis may give origin, within a single clone, to individuals of two diverse mating types, which thereupon conjugate together. This opened up the entire subject.

Comparative

The relations found in *Paramecium bursaria* and set forth in the present paper differ from those in *Paramecium aurelia* (SONNEBORN 1937, 1938) in the fact that in any race or group of the latter species there are but two mating types, conjugation being limited to union between these two. The situation thus resembles that in the higher animals and plants, with their two sexes. In *Paramecium bursaria* on the other hand, as set forth in the foregoing, each group contains four or eight mating types, the individuals of any one type being able to conjugate with individuals of any other type of the group. This occurrence of but two mating types in the one species, while another species of the same genus has several, is of great interest. Study of the genetics of *Paramecium bursaria* promises to throw light on the nature of this difference. In both *Paramecium aurelia* and *P. bursaria* there exist a number of different groups—three thus far discovered in each species—the individuals of any one group not conjugating with those of other groups.

In the flagellates Chlamydomonas, Polytoma and Protosiphon, according to the magnificent investigations of MOEWUS (1933, 1935, 1935a) there are in each species or race just two mating types, which unite in copulation. The flagellates are haploid, so that the cells of the two mating types are closely comparable to the male and female sex cells or gametes of the more complex animals and plants. MOEWUS considers the phenomena as strictly sexual, speaking of the two types as the two sexes. In Paramecium on the other hand the organisms are diploid, so that the different types are not directly comparable to the male and female gametes of higher organisms but only to the two types of somatic individuals that constitute the two sexes. Yet the situation in Paramecium differs from that in the typical bisexual diploids, in the fact that in the infusorian both members of the pair produce descendants.

In the flagellates, according to MOEWUS (1933) and others, the two sex types give off into the water diverse "sex stuffs"; by filtering or centrifuging the water containing these may be made free of the flagellate cells. Such watery solutions of the plus sex stuff induce temporary clotting when added to cultures of the minus sex, and vice versa. According to PASCHER (1931) in Chlamydomonas the active gametes of one sex orient about a quiet gamete of the other sex and from a distance swim directly toward it, the reaction being without doubt mediated by the "sex stuff" present in the water.

The situation in *Paramecium bursaria*, in which as many as eight different mating types exist in a single group, is obviously more complex for the action of sex stuffs dissolved in the water, than in the flagellates. Further,

the method of clotting and pairing described in the present paper does not seem to suggest the control of the reactions by sex stuffs dissolved in the water, as in the flagellates. No direct attempts to test the presence of sex stuffs and determine their possible role have as yet been made for *Paramecium bursaria*.

The many diverse mating types in *Paramecium bursaria* invite comparison with the facts of "multipolar sexuality" in certain of the fungi, notably in Ustilago and the Hymenomycetes. But in making such comparisons it must be held steadily in mind that the diverse mating types in Paramecium are diploid organisms, while the sexually reacting parts in the fungi are haploid. According to the account of BAUCH (1930), in Ustilago and presumably in Hymenomycetes a given local race has four diverse sexual types (haploid); these are seemingly determined by the combination of two pairs of factors, so that the four may be represented as AB, Ab, aB and ab. Mating among the four types occurs in accordance with definite rules depending on the similarity and diversity of the two factors present in each. The situation is such that usually a given type mates with only one other type out of the four that constitute the group—whereas in *Paramecium bursaria* each type mates with members of any of the other types of the group. In the fungi the four factors have in different races become modified in various ways, and these modifications alter the mating tendencies, so that there results a large number of diverse mating types. In principle and in details the situation in the fungi differs greatly from that in *Paramecium bursaria*.

The phenomena in *Paramecium bursaria* appear most naturally classifiable as "self-sterility," of the sort found in certain higher plants and animals. The single clone, like the single self-sterile plant, ordinarily does not fertilize itself: its cells do not unite in conjugation. But the single clone, like the single plant, may be fertilized by another; the cells of the single clone unite in conjugation with those of other clones, giving viable descendants.

But this is by no means the whole story, either in the infusorian or in the self-sterile plant. The farther phenomena in the two cases show many parallels in their details (compare the account of the general features of self-sterility in plants given by EAST 1929). In some cases the self-sterile plant becomes at times self-fertile, giving viable offspring; this is true also, as set forth earlier, for the single clones of the infusorian. Again, the phenomenon is not strictly one of "self" sterility merely, in either the plant or the infusorian. The single plant is sterile also with certain other plants, just as the single clone of *Paramecium bursaria* is sterile with certain other clones (those belonging to the same mating type). In most self-sterile plants the individuals can be divided into groups, such that

those belonging to the same group are infertile together, while those belonging to diverse groups are fertile together. Such groups are comparable to the different mating types shown in this paper to exist in *Paramecium bursaria*. In the infusorian the number of such types is definite and limited; there are four in each of Groups I and III, eight in Group II.

The phenomena in the infusoria, while showing many points of resemblance to those in the self-sterile plants, of course differ from them in certain respects, particularly in the mechanism or physiology of self-sterility. In plants this appears to be a matter of relative rate of growth of the pollen tubes on the stigmas of individuals of different types; in the infusorian there is merely a failure to unite in conjugation.

For the division of the infusorian species into groups that do not cross conjugate, I have not found parallels in the accounts of self-sterility in the higher plants and animals. Its real parallel appears to be the actual differentiation of members of a genus into species that do not cross. In most organisms the groups so differentiated differ in certain morphological and physiological characters as well. This may turn out to be the case, in very slight degree, with the groups of *Paramecium bursaria*. In that case, or perhaps even without such additional differences, the groups might be considered slightly marked cases of differentiation into species.

SUMMARY

1. Ordinarily, members of a single clone of *Paramecium bursaria* do not conjugate together.

2. But when members of two diverse clones are mixed, in some of the mixtures the members of the two clones quickly clump into large masses containing many individuals. From these clumps many individuals later emerge united into conjugating pairs.

3. The behavior of the individuals in clumping and mating is described in detail. There are two main factors in the mating process: (1) an active coordinated reaction of two (or more) individuals that come in contact; (2) a physical adhesion between the bodies of individuals that touch each other. This physical adhesion results in irregular and uncoordinated movements; to it is due the rapid clumping into large masses. The two factors may be in operation separately or together.

4. Clumping and pairing depend upon a number of different conditions, the most important of which is the maturity of the clones. For several weeks or months the clones derived from ex-conjugant individuals are immature; they do not clump or pair. The tendency to clump and pair arises slowly, being weak in early periods, so that but few pairs are formed; later it gradually increases. Diverse ex-conjugant clones of the same parentage vary greatly in the length of the period of immaturity.

5. Other important conditions for clumping and pairing are the nutritive condition of the individuals, and the time of day. Clumping and pairing occur mainly in the afternoon; not at all in the evening and during night; little in the early forenoon.

6. When two diverse clones are mixed, in some mixtures clumping and pairing occur, in others not. Individuals of clones that do not clump and pair when mixed are considered to belong to the same mating type; individuals of clones that do clump and pair when mixed are considered to belong to diverse mating types.

7. When among many diverse clones all possible mixtures of two clones are made, a number of diverse mating types are found to exist, having the following relations: individuals of any single type may clump and conjugate with individuals of any of the other types, but not with individuals of their own type.

8. Three separate groups of such mating types exist, the types of any one group not clumping nor conjugating with any types from the other groups. Group I contains four mating types; Group II, eight mating types; Group III, four mating types. Thus the species as a whole is thus far found to consist of 16 diverse mating types.

9. The occurrence and distribution of the 16 mating types of the three groups are described. Group I (four types) has thus far been found only in Maryland and Virginia; Group II (eight types) ranges from Massachusetts to California and from New York to North Carolina; Group III (four types) has thus far been found in North Carolina and in Massachusetts. Some of the types of a given group appear to occur more frequently than others.

10. The three groups appear to remain independent, not intercrossing.

11. Different clones, whether belonging to the same or different mating type or group, differ greatly in size, form and physiological characteristics, and these features may remain nearly constant in vegetative reproduction. Differences observed between representatives of different mating types or groups usually turn out to be merely clonal differences, not characteristic of the entire type or group. It is as yet not certain that the three independent groups show any other constant diversities, morphological or physiological.

12. A clone belonging to a certain mating type remains of that type for many months of vegetative reproduction (observed up to 18 months).

13. Yet very rarely self-fertilization occurs; single pairs are found among the members of a single clone or single mating type.

14. Some clones collected from natural habitats have never paired with any of the 16 mating types. Others refuse for long periods to pair, but eventually yield a few pairs with certain of the types.

15. Certain genetic relations are stated, but in the main these are reserved for later treatment.

16. The phenomena in *Paramecium bursaria* are discussed in comparison with those in (1) *Paramecium aurelia*, (2) flagellates, (3) fungi, and (4) particularly with the phenomena of self-sterility in higher plants and animals.

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A COMPARISON OF THE SALIVARY GLAND CHROMOSOMES OF DROSOPHILA MELANOGASTER AND D. SIMULANS*

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Received November 11, 1938

INTRODUCTION

THE general problem of chromosomes and speciation has interested cytologists and geneticists for a long time. The earlier studies on animals dealt with comparisons of the metaphase chromosomes, and evidence for homologies in related species was restricted to a similarity in chromosome number and to the appearance of the individual elements. Following MULLER'S X-ray work and the discovery of numerous chromosome rearrangements, the judging of homologies by comparing metaphase elements appeared hazardous because it became clear that very extensive gene realignments may occur without one's being able to detect them in the condensed chromosomes. In addition to this, the presence of inert chromatin (MULLER and PAINTER 1932) could make such comparisons very misleading.

PAINTER'S discovery and development of the salivary gland method for the study of chromosomes, opened a new era for the cytologist. He pointed out in his first paper (1933) that homologous chromosomes undergo somatic synapsis, and due to this phenomenon it is possible to compare in the most exact way the "active" or euchromatic areas in salivary gland chromosomes. In closely related species which can be hybridized one should be able to detect any chromosome rearrangements which might have occurred since the related forms originated from a common ancestor.

The salivary gland chromosome method has been employed thus far in the study of three species crosses. The first of these is the *Drosophila melanogaster* and *D. simulans* cross reported by PÄTAU in 1935. He found that with a few exceptions there is usually close synapsis in hybrids in the euchromatic areas. The distal ends of some of the chromosomes do not conjugate. There are two extra lines in the X chromosome of *D. simulans* which are not seen in *D. melanogaster*, and in the right arm of the third chromosome there is a long inversion, reported earlier by STURTEVANT (1929) who based his conclusion on genetic data. PÄTAU also noted that a part of the non-inverted proximal end of the right arm of the third chromosome failed to synapse and that the fourth chromosomes were entirely different and would not conjugate in hybrids.

* A part of the cost of the accompanying figures is met by the Galton and Mendel Memorial Fund.

KERKIS (1936, 1937) working with the same cross confirmed PÄTAU's findings, but failed to mention extra lines in the X chromosome of *D. simulans*.

The second species cross is *D. pseudoobscura* by *D. miranda* studied by DOBZHANSKY and TAN (1936). They showed that numerous and extensive changes have taken place since these species arose. Translocations and inversions are present in the X and in the second chromosomes. In the left limb of the X, and in the third, fourth, and fifth chromosomes, the gene arrangement is very different in the two species. Even in regions where the chromosomes exhibit similar patterns, the pairing of homologues is extremely variable.

The most recent study on hybrids is by METZ and LAWRENCE (1938), dealing with a Sciara cross, *S. ocellaris* and *S. reynoldsi*. They report that in the salivary glands of the hybrids, the chromosomes are associated in symmetrical pairs, but complete synapsis is found in only a few short regions. There is an absence of conspicuous translocation and inversion configurations, but a high frequency of small differences in corresponding regions, indicating that many small chromosomal changes have occurred in the recent evolution of the two species.

The present investigation was undertaken in order to make a very detailed study of the *D. melanogaster* and *D. simulans* hybrids, and if possible to determine the origin of the extra bands reported by PÄTAU for *D. simulans*. This work was carried out under the direction of PROFESSOR T. S. PAINTER, to whom the writer wishes to express his indebtedness and thanks.

MATERIALS AND METHODS

The salivary glands were dissected from mature hybrid larvae in RINGER'S solution (cold-blooded formula). Both temporary and permanent mounts were made. PAINTER'S acetocarmine method was used in the preparation of temporary mounts, and a modification of BRIDGES' aceto-carmine-alcohol-euparal method was employed in making permanent slides. All drawings were made using a 120× oil immersion lens, a 20× eye piece, and a camera lucida at table level.

The findings of this study can be presented best by describing the individual chromosomes. For the exact location of the bands BRIDGES' nomenclature is used.

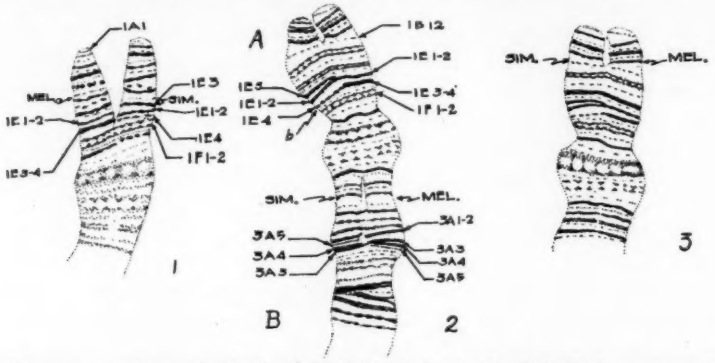
OBSERVATIONS

The X chromosome

In hybrids the proximal regions of the two homologues usually synapse intimately, but near the distal end there is a great deal of variability in the union due, mainly, no doubt, to the presence of several gene rearrange-

ments which are present here. Typically the tip ends lie separate, synapsis beginning at a line 1 F 1–2, just to the left of the puffed area (fig. 1). In some cases, however, the ends are joined up to and including lines B 12 or 13 (fig. 2), but in no case has synapsis been found beyond this point, although the bands appear alike up through the line 1 A 2 (fig. 2).

Just at the tip of the free left hand end, PĂTAU has reported that in *D. simulans* the band 1 A 1 is missing. This region has been examined with great care, and it is true that often in hybrid larvae an extra band appears in *D. melanogaster* as in figure 1. However, it is extremely difficult to determine the exact pattern in these ends, and the absence of a band in *D. simulans* does not rest on a very secure basis.



FIGURES 1–3.—Camera lucida drawings of the left hand ends of the X chromosomes of a hybrid larva. Figure 2 shows the presence of two short inversions in the region of the 1 E 1–2 band and at the 3 A 1–2 level.

Figure 2 is made from a cell of a hybrid larva in which synapsis is unusually complete. The *melanogaster* chromosome lies to the right, and the *simulans* component to the left. At the point marked A in the figure it will be noted that the *melanogaster* band pattern consists of two doublets, 1 E 1–2, and 1 E 3–4, the latter being about twice as broad as the former. In the *simulans* chromosome, the band 1 E 1–2 is continuous with its *melanogaster* homologue, but a broad 1 E 3–4 band is not seen. Instead a rather narrow band, labeled provisionally 1 E 4 is synapsed with the 1 E 3–4 band of *melanogaster*. To the left of the 1 E 1–2 band in *D. simulans* there is an extra band labelled 1 E 3 which is about equal in breadth and staining intensity to line 1 E 1–2. Essentially the same features are brought out in figure 3. Note that in each case the line 1 E 1–2 synapses in the hybrid, and that a narrow band of *D. simulans* (1 E 4) is joined with the broad 1 E 3–4 band of *D. melanogaster*. The explanation for the difference of patterns in the two species is that in *D. simulans* the bands 1 E 1–2 and

1 E 3 are inverted so that 1 E 3 lies to the left of the former. PAINTER and GRIFFEN (1937) have indicated that "double" bands may be made up of one, two, or more rows of chromomeres, and although BRIDGES (1938) represents the lines 1 E 3–4 as equal sized, we are forced to conclude that we are dealing here with a band involving at least two rows of unequal-sized chromomeres, since in *D. simulans* the band 1 E 3 is twice as broad as 1 E 4. The extra band reported by PÄTAU for *D. simulans* is, of course, the 1 E 3 band which lies separate in this species.

It is a point of unusual interest to note that although the band 1 E 1–2 in *D. simulans* is inverted, it synapses perfectly with its uninverted homologue in *D. melanogaster*.

From A to B in figure 2, there is a similarity in the patterns of the two species and synapsis is often complete through the band 3 A 1–2. In *D. melanogaster* next to the broad band 3 A 1–2, there are two broad bands 3 A 3, and 3 A 4, and then a narrow one 3 A 5. In *D. simulans* the order is reversed and bands 3 A 4 and 3 A 5 lie to the left of band 3 A 3. The 3 A 3 bands, even though one of them is inverted, often synapse in hybrids and cause a certain amount of distortion as a result (fig. 2). On the other hand, bands 3 A 4 and 3 A 5 being out of position in *D. simulans* do not unite in the hybrid. Here again the extra band reported by PÄTAU in *D. simulans* is a result of an inversion.

Farther to the right along the X chromosome in hybrids, certain regions characteristically fail to undergo intimate synapsis, namely regions 7 C, 10 B and F, 12 D to F, and 18, 19 and 20. But in each instance cells have been found in which synapsis is completed, indicating that these regions are similar in nature in the two species.

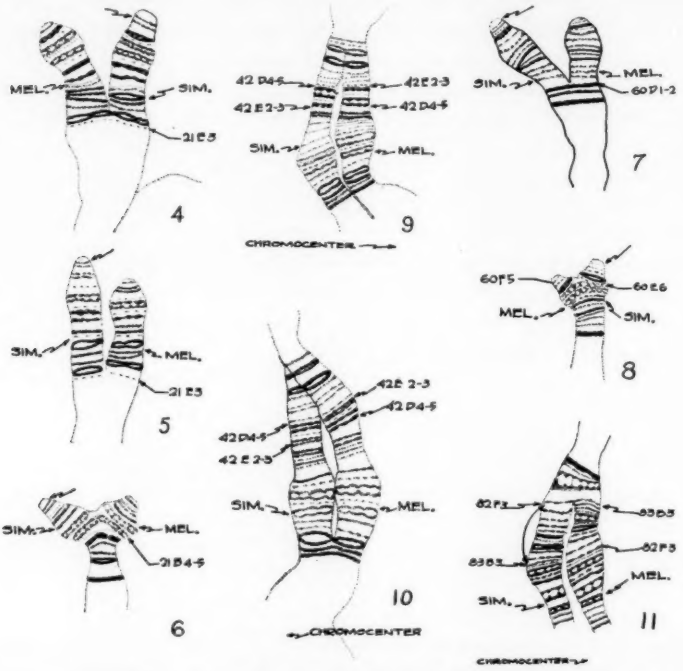
Second chromosome

As KERKIS has pointed out, the distal ends of the left arm of the second chromosome never completely join in synapsis. Typically the union ends at about the band 21 E 3 (figs. 4 and 5) but in exceptional cases may extend to 21 B 4–5 (fig. 6). There is a consistent tendency for the *melanogaster* chromosome to be shorter and thicker at the end region, and for the *simulans* component to be extended, especially the extreme tip. Otherwise there is no detectable difference in the band pattern of the two species until the free tips are reached. Here *D. simulans* shows one band that can not be seen in *D. melanogaster* (figs. 4 and 6).

In regions 33, 34, 35, 38 and 39 in the left arm conjugation is usually incomplete, but in all instances cells have been found in which synapsis is completed and we have no morphological evidence for any changes in these regions for the two species except their failure to synapse regularly.

The free end of the right arm of the second chromosome behaves very

much like the tips of the left arm. Figures 7 and 8 show the normal range of synaptic union. It will be observed that the *simulans* chromosome has a tendency to be more elongated than the *melanogaster* component and again we see in the figures the presence of an extra band in *D. simulans* right at the tip end.



FIGURES 4–11.—Figures 4–6 show the range of synaptic union in the distal end of II L and the presence of an extra band in *D. simulans*. Figures 7 and 8 are from the distal end of II R. Figures 9 and 10 show the presence of a short inversion in the right arm of chromosome II near the chromocenter region. Figure 11 shows the presence of a short inversion in the right arm of chromosome III near the chromocenter.

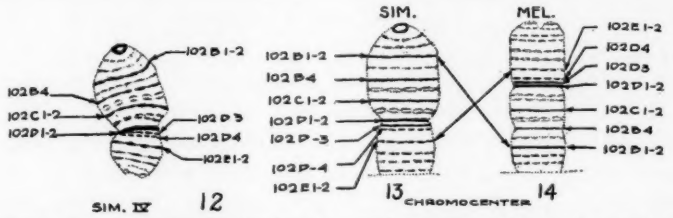
Although there are short areas along the right arm which frequently do not join in synapsis, notably at 58 D and E, no other evidence of a change in pattern or of homology has been found until the region of the spindle fiber is reached. Here at region 42 (figs. 9 and 10) there is a very short inversion in *D. simulans* which involves bands 42 D 4–5 to 42 E 2–3. As often happens, even though the inversion is quite short, the chromosomes in hybrids usually remain apart for some distance on either side of the rearrangement.

Third chromosome

The left arm of the third chromosome shows complete synapsis in hybrids and no differences in band pattern have been found. The distal end of the right arm also appears to be similar in the two species. There are, however, two inversions in the right arm of the third chromosome. A large inversion (described by PÄTAU) extends from band 84 B 3 to band 92 C 3. A second inversion, which is relatively small, is nearer the spindle fiber end and is not quite so obvious (fig. 11). As indicated in the figure the inverted section of *D. simulans* extends from 82 F 3 to 83 B 3 on the *melanogaster* chromosome, and involves some ten or twelve bands.

Fourth chromosome

The outstanding features of the fourth chromosome of *D. simulans* (figs. 12 and 13) are: A rather narrow basal portion next to the chromocenter which is limited distally by three deeply staining bands, 102 D 1–2,



FIGURES 12–14.—Figure 12 is a drawing of a normal chromosome IV taken from *D. simulans*. Figures 13 and 14 are semidiagrammatic drawings of the fourth chromosomes of *D. melanogaster* and *D. simulans* and the arrows indicate the *minimum* limits of an inversion present in the latter species.

3 and 4. The distal two-thirds of the chromosome is typically much broader than the basal third, and ends in a deeply staining ring. In contrast to *D. melanogaster* the distal end of the chromosome has never been observed attached to the chromocenter. The salient features of the fourth chromosome of *D. melanogaster* are shown diagrammatically in figure 14. Two banded areas are conspicuous. The first lies near the chromocenter and consists of the deeply staining band labeled 102 B 1–2. The second is made up of three bands (102 D 1–2, 3 and 4 which are found about two thirds of the length from the chromocenter. Arrows in figures 13 and 14 indicate the minimum limits of an inverted area in *D. simulans*. Starting with the band 102 B 1–2 in both species and reading in opposite directions, the figures show that we have exactly the same sequence of band pattern in reverse order. In *D. simulans* the homologies of the lines beyond the arrows, at either end, are uncertain because somatic synapsis has never been observed here in hybrids. In *D. melanogaster*, there are two distinct

bands between line 102 B 1–2 and the chromocenter. In *D. simulans* there are three bands of similar breadth and intensity of staining. At the distal end of the *melanogaster* chromosome there are four distinct bands and the nipple-like ends with their faint lines. In *D. simulans* there are only two distinct lines beyond 102 B 1–2, and the ring-like terminal band has no obvious homologue in *D. melanogaster*. It is quite possible that the inversion in *D. simulans* involves bands beyond the points indicated by the arrows, and thus that the three bands next to the chromocenter in *simulans* are really 102 E 3, 4, and 5. If this is true, then the two bands next to the terminal ring of *D. simulans* have their homologues in the basal part next to the chromocenter in *D. melanogaster*. In any event, we are forced to conclude that in addition to a relatively long inversion, other changes have occurred in the fourth chromosomes of the two species which have affected the distal ends. The origin of the terminal ring in *D. simulans* is obscure.

DISCUSSION

The present study has given evidence of at least ten gene rearrangements which have occurred in the chromosomes of *D. melanogaster* and *D. simulans* since they arose from a common ancestor. Of these rearrangements, six are demonstrable inversions (five hitherto unreported) and four involve changes in the band pattern at the distal ends of certain chromosomes.

The five newly described inversions are all small, involving two (one and a part of a doublet), three, three, ten and ten fairly conspicuous bands respectively. The very faint lines are not considered here. This raises the very interesting question of how such small inversions could have been produced. On the basis of SEREBROVSKY'S hypothesis, at first sight, it is difficult to visualize loops in the chromonema thread so short as to involve only two or three chromomeres, as this would seem to imply that the thread was greatly elongated and tangled when the lesions producing the change occurred. This difficulty, however, is largely removed if we assume that in animals, as in plants, the chromonema is coiled in a minor spiral as well as in the more easily observed major spiral. The minor spiral coils would favor small intrachromosomal rearrangements for, due to the minor coiling, adjacent regions of the chromonema would be in close physical contact. On this basis we would expect that small rearrangements would be the rule and this seems borne out in the present study as well as by the work of DOBZHANSKY and TAN dealing with *D. pseudoobscura* and *D. miranda* and the Sciara cross of METZ and LAWRENCE.

Changes in the free ends of the salivary gland chromosomes are considerably more difficult to determine exactly, than in the body of the

chromosome, and for this reason we cannot be so sure in interpreting our observations. Nevertheless, it seems clear that in *D. melanogaster* there are three bands of moderate breadth and intensity (one extra band in the X and two extra bands in the fourth chromosomes) not seen in a corresponding position in *D. simulans*. Conversely in *D. simulans*, there are three bands (one extra band in the left arm of the second, one in the right arm of the second, and the terminal ring on the fourth chromosome) not observed in a corresponding position in *D. melanogaster*. The three extra bands of *D. melanogaster* are offset by the three extra bands of *D. simulans* and except for changes in the tip of the fourth chromosome which probably have another explanation, no evidence has been found that there has been the loss or the addition of any band in either species.

The consistent failure of the tip ends of the X and the second chromosomes to synapse in hybrids would seem to suggest that mutual translocations have occurred involving the X, the left and the right arms of the second, and the fourth chromosomes. But this would scarcely explain the relatively deeply-staining ring at the end of the fourth chromosome in *D. simulans*. Regarding the latter, it will be recalled that whereas the distal end of the fourth chromosome in *D. melanogaster* is connected with the chromocenter, in *D. simulans* the free tip has not been observed behaving in this manner, and the ring may have been produced in some way when the fourth chromosome became disconnected from the inert chromatin, or became connected with it.

One of the most interesting features brought out in this study is the fact that the inversion of a band does not prevent it from synapsing in its reversed position directly with a homologous band in another chromosome, and thus it is clear that somatic synapsis does not depend entirely on the orientation of the band in the chromosome. On the other hand, a very short inversion, involving two or more bands ordinarily disturbs somatic synapsis for some distance on either side of the rearrangement. The change in some way seems to interfere with a complete union of the adjacent bands. The nature of this effect is unknown.

The fact that an inverted band occasionally will synapse with its homologue would effectively prevent us from determining "one band" inversions. From morphological observations, the only indication that an inversion has occurred would be the frequent failure of a limited area, along a chromosome, to synapse. Both PÄTAU and KERKIS have indicated several such regions in *D. melanogaster* and *D. simulans* hybrids, and we have recorded above a considerable number of such areas, where the morphological pattern is the same in both chromosomes, but usually the chromosomes lie separated for a short distance. Exceptionally the union is complete. It seems probable that this failure to synapse regularly is due to

some undetected rearrangement, perhaps the inversion of a single band, or to a change in some extremely narrow and lightly staining line.

There are altogether some 14 regions (seven in the X, five in the left arm and two in the right arm of the second chromosomes), where the visible morphological pattern of the bands of homologues are similar and yet do not ordinarily undergo somatic synapsis. If we grant that a consistent failure to synapse is *prima facie* evidence for a change in the band pattern, and add these 14 areas to the ten regions of demonstrable changes, we get a total of some 24 chromosomal rearrangements which have occurred in *D. melanogaster* and *D. simulans* since they arose from a common stem. Of these changes only one involves many bands.

SUMMARY

1. In the salivary chromosomes of hybrid larvae between *D. melanogaster* and *D. simulans*, ten clear chromosomal rearrangements have been found. Six of these are inversions, five of which are very short, and four involve changes of one or a few bands at the free ends of certain chromosomes. In addition to these demonstrable rearrangements, there is a total of 14 short areas where the chromosomes do not synapse ordinarily.

2. Very short inversions show that an inverted band will occasionally synapse directly and intimately with its homologue, thus proving that synapsis is not entirely dependent upon the band's orientation. This fact indicates that single band inversions can not be detected cytologically.

3. Ordinarily, a very short inversion prevents somatic synapsis for some distance on either side of the rearrangements, and it seems probable that the 14 short areas where somatic synapsis does not usually occur, are due to cytologically undetectable rearrangements.

4. The evidence suggests that as many as 24 rearrangements have occurred in the chromosome of *D. melanogaster* and *D. simulans* since these arose from a common stem.

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THE EFFECT ON DEVELOPMENT OF A LETHAL DEFICIENCY IN DROSOPHILA MELANOGASTER: WITH A DESCRIPTION OF THE NORMAL EMBRYO AT THE TIME OF HATCHING[1]

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Received November 15, 1938

INTRODUCTION

BECAUSE, as a rule, lethal genotypes have their effect during embryogeny, where the tempo of development and the magnitude of the structural changes are most marked, they are particularly useful in investigating the role of the genes in development. Furthermore, a study of early-acting lethals offers the possibility of increasing our knowledge of the normal embryology of forms about which little is known at present.

Investigations of the nature of lethal disturbances have involved two questions: 1) whether the effect can be primarily ascribed to a single structure or group of structures which, because of their pathological condition, prevent further development, or; 2) whether the effect appears to be of a general character, expressed as a disturbance of the dynamics of development which may lead to malformations.

The study reported below deals with a deficiency of the X chromosome in *Drosophila melanogaster* which is lethal when hemizygous. This lethal is interesting because it terminates development at the very end of the egg stage, thus affording an opportunity of studying organs and anlage which are already well differentiated.

Since the normal animal at this stage has not been described in any detail in the literature, an account of the developmental phenomena, and of the anatomy of the larva just prior to hatching has been included for comparison with the lethal embryos.

MATERIAL AND METHODS

The deficient X chromosome is one of a series produced by means of X-rays by DEMEREC during his study of "cell-lethals" (DEMEREC 1934a, 1934b, 1935, 1936; DEMEREC and HOOVER 1936). It is not cell-lethal but is zygote-lethal when hemizygous. The chromosome is designated as deficiency 260–2 by DEMEREC (1937), and carries the dominant marker Bar.

[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University.

Part of the investigation was carried on at the Marine Biological Laboratory, Woods Hole, Massachusetts, under a grant from the Dyckman Fund by the Department of Zoology, Columbia University.

In the salivary glands of larvae heterozygous for this deficiency, DEMEREC and HOOVER (1936) found that the left-most eight bands of the deficient X were missing. Genetic tests indicated that the loci for yellow and achaete, but not for scute, are missing. At least four known loci are probably missing, namely: chlorotic, lethal J1, yellow and achaete. The locus for Hairy-wing (0.0+) may also be missing.

The stocks obtained from DR. DEMEREC, had the deficient chromosome balanced by the inversion dl 49 *y Hw* $m^2 \cdot g^4$. During the later stages of the investigation, the loci yellow and Hairy-wing were eliminated from the original dl 49 chromosome by a single crossover with a wild-type X from an Oregon-R stock. The experimental stock as maintained at present is thus (*y ac*)$^-$ *B*/dl 49 $m^2 \cdot g^4$ ♀ ×dl 49 $m^2 \cdot g^4$♂.

Three types of adults are present in these cultures: 1) (*y ac*)$^-$ *B*/dl 49 $m^2 \cdot g^4$ females which are fully fertile; 2) homozygous dl 49 $m^2 \cdot g^4$ females, which are partially sterile; and 3) dl 49 $m^2 \cdot g^4$ males, which are fertile. No male imagoes which might be construed as bearing the deficient X have appeared in any of the cultures (ca. 30,000 flies). Such males, if viable, would be expected to be yellow and achaete, for MULLER (1935) got adult males bearing an X deficient for the loci yellow and achaete, and described these as phenotypically yellow and achaete.

Egg collection methods

For egg-viability counts to determine the stage at which death occurred, matings of single (*y ac*)$^-$ *B*/dl 49 females to two or three males of a different stock (inbred Oregon-R wild type in one series, and white males in another) were made. Egg deposition took place on waxed paper spoons containing a slab of 2 percent agar dissolved in about 20 percent molasses. Collections of eggs were made every 24 hours, and the number laid recorded. The eggs were then kept at 25°C for another 24 hours, at which time a count was made of the number that had failed to hatch. From previous experience of BREHME (1937) and the author, it was known that the modal hatching time of normal eggs is 20–22 hours after laying (25°C) and that practically all normal eggs have hatched by 24 hours. In several cases check counts were made 48 hours after the eggs had been collected, and no additional eggs were found to have hatched.

For histological purposes, and for a study of the live eggs, a mass egg collection method was used. This was a modified version of the technique described by SCHWEITZER (1936). The above food medium was poured on to paper bottle caps. About 40 pairs of flies in a half-pint milk bottle were allowed to oviposit on the caps for two hour periods. If the females had been laying eggs actively previous to the collection, the degree of development for any one batch of eggs was fairly uniform.

Observations on live material

As has been indicated by HUETTNER and RABINOWITZ (1933), CHILD and HOWLAND (1933), and POULSON (1937b), the living egg lends itself readily to observation. This is particularly true of the later stages, when much of the opaque yolk has been assimilated. To observe the eggs, the translucent chorion must be removed. The technique employed for this operation is essentially the one described by POULSON (1937b). The dechorionated eggs are placed in a drop of Ringer's solution on a glass slide and the preparation is sealed with a supported cover-slip whose edges have been "vaselined." Some air is enclosed under the cover. Such preparations will hereafter be referred to as "wet mounts." The eggs are immersed in the liquid and, as a rule, lie at the liquid-air interface. These preparations can be observed with the highest powers of the microscope, a 100× oil-immersion objective having been successfully employed. Normal larvae have been observed to hatch from the eggs and remain alive in such preparations for as long as four days after hatching.

Histological techniques

Studies of the anatomy and histology of the late eggs were made both from sectioned material and dissections. Cross-sections, longitudinal and frontal sections were cut at 7μ for the very early stages, and at 5μ for the later stages.

As HUETTNER (1923) has shown, the egg membranes must be punctured to obtain satisfactory penetration of the fixing fluids. Steel needles ground to a fine point were used for this purpose.

Fixatives employed were Kahle's fluid (F.A.A. of HUETTNER), San Felice's fluid, Bouin's, and Allen's B-15 modification of Bouin's fluid. Kahle's fluid is very satisfactory for the early stages, and San Felice's fluid is excellent for the very late stages.

The material is dehydrated in the usual manner, passed through cedarwood oil and benzol, and embedded in hard paraffin of 56°–58°C melting point.

Heidenhain's haematoxylin and gentian violet were used as nuclear stains. Gentian violet was found to be by far the more satisfactory of the two. The nuclear material differentiates sharply if the stain is properly manipulated (see NEWTON and DARLINGTON 1929). An effective counterstain is obtained by employing a solution of 0.02 percent eosin in 95 percent alcohol as the first alcohol in the series used to differentiate the gentian violet. Light green was used as a counterstain for the iron-haematoxylin.

Total mounts of dechorionated eggs were also prepared, using the Feulgen reaction. For the later embryonic stages these preparations have little

advantage over the living material for general topographic studies, but they are excellent for the early stages, where the large amount of yolk is an obstacle to clear optical definition in the living egg.

To get a successful preparation it is best to treat the material with cold N/1 HCl for about 25 minutes before immersion in the hot hydrochloric acid. The reaction with the fuchsin-sulphurous acid dye must be followed by three changes of SO_2 water, 15 minutes in each change, and subsequent adequate washing in distilled water and the graded alcohol series.

All experiments were conducted at a temperature of $25 \pm 1°C$, unless otherwise stated. The age of the animals, both normal and deficiency-bearing, is computed throughout this paper in hours from the time the eggs were laid, unless otherwise indicated. As a rule, the stated age should be accurate to within one hour. The deficiency-bearing larvae will be referred to as the "mutant" animals.

DETERMINATION OF THE STAGE AT WHICH DEATH OCCURS

If death of the animals hemizygous for the deficiency takes place during the egg stage, at least 25 percent of the eggs should fail to hatch.

DEMEREC and LEBEDEFF (1935) concluded from egg and larval counts of the same deficiency that death occurs exclusively during the larval period. DEMEREC and HOOVER (1936) concluded from similar observations that death occurs both in the egg and larval stages. The latter authors found that 16.9 percent of the eggs from def. 260–2/ dl 49 *y Hw* $m^2 \cdot g^4$ females failed to hatch, while in the controls *rux*/ dl 49 *y Hw* $m^2 \cdot g^4$ females 7.3 percent were inviable. The total egg-larval mortality for the experimentals was 33.4 percent and 18.9 percent for the controls. No pupal mortality figures were given. Their data thus indicate that the lethal takes effect in both egg and larval stages and possibly in the pupal stage.

In the present case, of a total of 5,748 eggs laid by 28 females, 1,467, or $25.5 \pm .0057$ percent, failed to hatch. Control figures from other experiments for the "residual inviability" of normal stocks (STURTEVANT and BEADLE 1936; SCHWEITZER and KALISS 1935; BREHME 1937; KALISS, unpublished) have a range of from three to eight percent. The present experiment then left open the possibility that from three to eight percent of the deficient eggs hatched.

From the above data no certain conclusion can be drawn as to the time of death of the mutants. It is clear that a purely statistical analysis is unsatisfactory in this case. The difficulty was obviated in the present instance by a method of distinguishing the mutant genotypes *phenotypically*.

It had been noticed that the eggs that failed to hatch contained larvae with *yellow* mouth parts, and cuticular spines that were more lightly pigmented than those of the wild type. Of those that did hatch, some of

the first instar larvae had yellow mouth parts, and some black. The distinction was sharp. (The mouth armature and cuticular spines of genotypically *yellow* larvae and late embryos is also "yellow-type" as contrasted with the darker pigmentation in wild-type animals.)

Now since the matings used to collect eggs were $(y\ ac)^{-}B$/dl 49 *y Hw* $m^2 \cdot g^4$ ♀ × + ♂, the larvae obtained should consist of 50 percent females which are wild type phenotypically with respect to pigmentation, 25 percent males which are yellow type genotypically and phenotypically, and 25 percent males which are deficient for the yellow locus and might be expected to be phenotypically yellow-type, since the yellow mutation is known to be hypomorphic (MULLER 1932, 1935).

A stock of flies was then synthesized containing $(y\ ac)^{-}B$/dl 49 $m^2 \cdot g^4$ ♀ ♀ and dl 49 $m^2 \cdot g^4$ ♂ ♂, the loci for yellow and Hairy-wing having been eliminated from the original dl 49 chromosome by a single crossover with a wild-type X. The larvae from this mating should all be phenotypically wild type, with the possible exception of the mutant larvae which might be "yellow-type." Actually all the larvae that *hatched* had a wild-type (black pigmented) mouth armature and cuticular spines. Those that failed to hatch had a "yellow-type" mouth armature and cuticular spines and presumably were the mutant males.

During the course of mass egg collections for histological purposes 410 first instar larvae were classified as to the color of their mouth armature. They were all non-yellow, indicating that no mutant larvae had hatched. MULLER's classification of the yellow locus as hypomorphic is thus substantiated for the larva by the phenotypic effects of its absence. The fate of the hatched larvae was not followed.

TABLE 1

Determination of the stage at which death occurs. $(y\ ac)^{-}B$*/dl 49* $m^2 \cdot g^4$ ♀ ♀ *mated to Oregon-R*+♂♂.

♀	NUMBER OF EGGS LAID	NO. OF EGGS HATCHED	NO. 1ST INSTAR LARVAE RECOVERED	% EGGS UNHATCHED	NO. UNRECOVERED LARVAE
1	175	139	139	20.6	0
2	206	150	150	27.2	0
4	237	179	178	24.5	1
5	309	224	221	27.5	3
6	292	218	218	25.3	0
7	184	133	133	27.7	0
8	341	235	235	31.1	0
9	281	219	217	22.1	2
10	161	124	124	23.0	0
Total	2186	1621	1615	25.8	6

All the larvae recovered had wild-type (black) mouth armature.

As a result of these observations, a second series of egg counts was made, a record being kept of the number of eggs that hatched, and the number and phenotypes of the first instar larvae recovered (table 1). From nine pair matings of females of the constitution $(y\ ac)^{-}B/$ dl 49 $m^2 \cdot g^4$ mated to Oregon-R wild-type males 2,186 eggs were obtained. Of these, 565, or 25.8 percent failed to hatch. Of an expected 1,621 hatched larvae, 1,615, all phenotypically non-yellow, were recovered. Six larvae were not accounted for. No yellow-type larvae hatched. It thus appears that extremely few, if any, of the mutant larvae hatch under the conditions of our experiments.

THE NORMAL LARVA SHORTLY BEFORE HATCHING

PARKS (1936) and POULSON (1937b) have laid the groundwork for an embryology of *Drosophila melanogaster*. A bibliography of Drosophila literature on this subject will be found in POULSON's monograph.

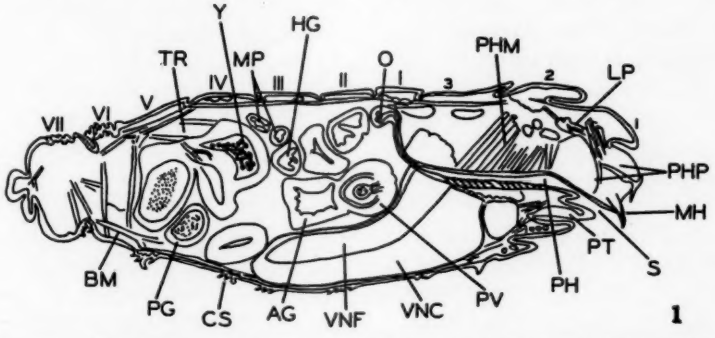


FIGURE 1.—Semi-diagrammatic drawing of a mutant 22±2 hours old. Slightly parasagittal section. Magnification, 214×. For abbreviations used see p. 256.

POULSON (1937b) has described the differentiation of the larval organs up to the late egg stage but the details of this stage still have to be completed. An account is therefore given of the late embryo about two hours before hatching (approximate age 20–22 hours). As POULSON states, the larva is essentially complete by this time. The eggs used were obtained from a long inbred Oregon-R stock. They were observed as wet mounts, and also dissected.

Examination of these preparations under magnifications of 150× and 660× revealed the following:

Characteristic landmarks of the alimentary tract are the long, parallel fibers of the pharyngeal dilator muscles (fig. 4 PHM), and the pear-shaped proventriculus. The tract can be traced with ease almost in its entirety,

the exception being the much-coiled midgut. Portions of the latter are filled with yolk globules which show a gradation in size with their progressive digestion, being smallest in the posterior portion of the midgut (fig. 8 Y). Active peristalsis is present throughout the length of the tract.

The fully developed mouth armature (fig. 2 MZ; fig. 1: LP, MH) is pigmented a pale gray, a characteristic which is already at this stage a useful genetic character (KALISS 1937b).

The first appearance of gas in the tracheae, to be described in detail below, occurs at this time. Coincident with this event is a marked increase in the activicity of the embryo and the first appearance of irregularly shaped concretions of fairly uniform size in the posterior Malpighian tubules. The process of accumulation of these concretions, which are presumably calcium carbonate (see KEILIN 1921), is rapid, so that by the time of hatching the posterior tubules have a characteristic white color due to the milky fluid in the lumen.

The anterior pair of tubules, with the exception of the distal portions clearly shows the yellow color described by BEADLE (1937). The pigment is present as discrete yellow globules in the cytoplasm of the epithelial cells (KALISS 1937a). It is also present in the posterior pair of tubules throughout their length, but is more difficult to see because of the milky contents. The atrial portions of both pairs of tubules show active peristalsis (EASTHAM 1925).

(It is a point of interest that with respect to the contents of the tubules, the first instar larva differs from the second and third instars. The posterior tubules of the newly hatched larvae already contain milky suspensions and continue to accumulate concretions during the course of the first instar. The distal portions of the anterior tubules also fill, *after hatching*, with milky suspensions which contain small spheres of fairly uniform diameter. In the second and third instars (BEADLE 1937; EASTHAM 1925) the distal portions of only the anterior tubules are filled with spherical concretions of a variety of sizes, while the posterior tubules have occasional concretions which are often irregular in shape.)

The body integument (cuticle) is covered with spines (figs. 1 and 8: CS). Those on the ventral surface are pigmented. This pigmentation is another useful larval character, since in genetically *yellow* larvae of this age the spines are either very lightly pigmented or not at all, in contrast to their marked pigmentation in the non-yellow larvae.

The head segment and the last abdominal segment carry sensory papillae. Their distribution corresponds to the description given for them in older larvae by HERTWECK (1931), and for *Rhagoletis pomonella*, the apple maggot, by SNODGRASS (1924).

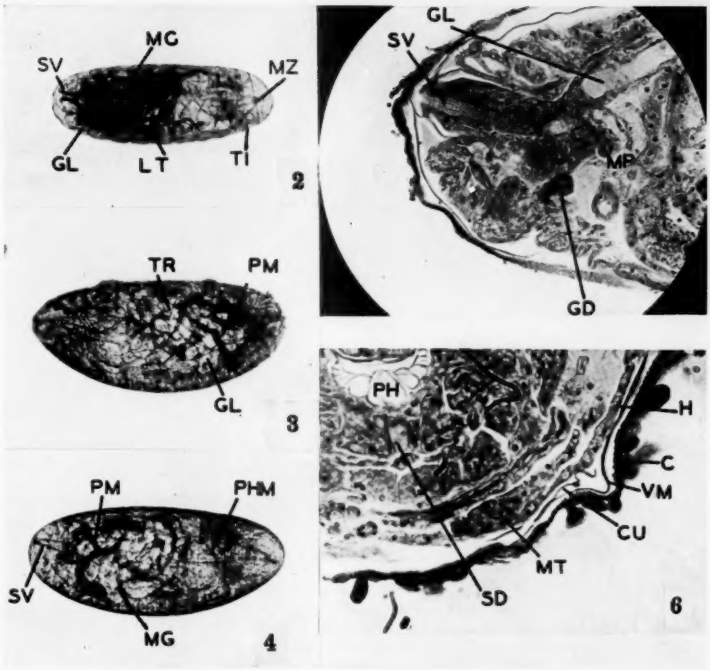


FIGURE 2.—Dorsal view of wild-type Oregon-R larva enclosed in vitelline membrane. Egg dechorionated and mounted in paraffin oil. Photograph of live animal taken shortly after gas has spread through the tracheae. Gas has not spread completely through the anterior portion of the right great lateral trunk. 73×.

FIGURE 3.—Live mutant about 24 hours old, enclosed in vitelline membrane. Slightly oblique dorsal view. Gas present in finer tracheal branches, but not in great lateral trunks. 80×.

FIGURE 4.—Live mutant about 24 hours old, enclosed in vitelline membrane, showing the heavily charged posterior Malpighian tubules. No gas in the tracheae. Lateral view. 80×.

FIGURE 5 (upper right).—Sagittal section of a mutant 22±2 hours old. Posterior end. 293×.

FIGURE 6.—Cross section through the mesothoracic segment of a wild-type egg 22±1 hours old. 513×.

The first appearance of gas in the tracheal system of the normal larva

Since one of the marked differences between the mutant larva and the normal at 22 hours, that is, shortly before hatching, was found to be in the extent of the spread of gas in the tracheal system, a detailed study of the first appearance of gas was undertaken. We are not aware of any previous accounts in the literature of this phenomenon in Drosophila. For this purpose normal eggs 18 hours old were dechorionated and observed under the 16 mm and 4 mm objectives in wet mounts.

In the few cases observed (six eggs), the first bubble of gas appeared in one of the great lateral trunks, right or left, either immediately in front of the spiracular vestibule or near the middle of the trunk. Once the bubble appeared, it rapidly increased in size, displacing the fluid in the tracheae (fig. 2: GL, LT). In three cases where it could readily be followed because of the favorable orientation of the animal, the gas was seen to spread across the posterior large commissure to the trunk on the opposite side, and down that trunk anteriorly. The gas spread caudad in the spiracular vestibules toward the spiracles, first replacing the fluid in the central axis and then filling the spaces among the dense network of hairs lining the vestibule. It spread anteriorly and laterally along the segmental branches and small dorsal commissures in discontinuous jumps. The tracheal system was filled in regular order, the gas filling the tubes of larger diameter before entering those of smaller diameter. Thus the posterior segmental branches, which are larger than the anterior segmental branches, filled first, the branches in each segment filling in sequence from posterior to anterior. Likewise, the spiracular vestibules, which are of smaller diameter than the great trunks into which they lead, filled with gas after the great trunks.

The latter observation indicates that the fluid is absorbed through the tracheal integument throughout the length of the system.

The time for the whole process was short, the great lateral trunks filling in three to four minutes, while the whole system filled in about 10–15 minutes. With the displacement of the fluid the helical folds in the tracheal cuticle, the taenidia, became apparent due to the change in refractive index. The animals hatched two to two and a half hours later.

It has been observed in the eggs of several species of insects that gas will spread through the tracheal system before hatching, and while the animal is still surrounded by the amniotic fluid. This phenomenon was first described by WEISMANN (1863) in the embryo of *Musca* (=*Calliphora*) *vomitoria*. KEILIN (1924) reviews the literature on this point in apneustic insects. SIKES and WIGGLESWORTH (1930) describe the spreading of gas before hatching in the grain moth (*Sitotroga cerealella*) and in the

blow-fly (*Lucilia sericata*). In Sitotroga there is no gas in the stigmatic chambers before the animal hatches. In Lucilia, gas appears in the great tracheal trunks, but spreads to the finer branches after the larva hatches.

The events in Drosophila and in the forms just described contrast with those in insects in which gas appears only after the surface of the larva has dried while it is still enclosed in the egg membranes (Ceratophyllus, Tenebrio); or in those larvae which must free themselves of all embryonic membranes before air invades the tracheae (Cimex, Polyplax) (SIKES and WIGGLESWORTH 1930).

WIGGLESWORTH (1930, 1931a, b), investigating the movement of fluid in the terminal portions of the tracheoles in the mosquito larva and several terrestrial insects, has shown that there is a direct correlation between the degree of muscular activity and the distance to which air will penetrate the tracheoles supplying the specific muscles observed. He argues that the metabolites resulting from the increase in muscular activity increase the osmotic pressure of the muscles and haemolymph and consequently cause absorption of the fluid from the tracheoles supplying them.

SIKES and WIGGLESWORTH propose that this theory can be applied to the observed differences in the time of appearance of gas in the various types of insect eggs if the fact be taken into account that in the case of the Dipteran and Lepidopteran eggs described above the animals become extremely active just at about the time the gas appears.[2]

Hatching activities of the normal larva

Another striking difference between the normal and mutant larva is in the degree of activity inside the vitelline membrane shortly before what would be the hatching time of the normals. The normal larva, commencing about two to two and one-half hours before hatching, is in constant and vigorous motion. The anterior end of the animal is continually striking and rubbing against the confining vitelline membrane. At the same time, the mouth hooks are exploring the membrane, occasionally entering the micropyle and, thereby having gained purchase on the membrane, pulling it back by retraction of the hooks. At times a dorsal plate between the two hooks, the "median tooth" which is present only in first instar larvae (M. STRASBURGER 1932), is extruded and pushed against the membrane.

[2] Since this has been written, two papers by WIGGLESWORTH (1938a, b) have appeared bearing on the question of the extension of gas through the tracheal system of mosquito larvae. The data indicate that the "nervous system is apparently concerned in the initiation" of absorption of the tracheal fluid. Also "reasons are given for doubting that the level of fluid in the tracheoles is determined by a *direct* relation between capillarity and osmotic pressure of the blood." " . . . it appears as though the extent to which fluid rises in the tracheoles is dependent upon the interaction of several forces: the osmotic pressure of the body fluid, the swelling force of the cytoplasm around the tracheoles and perhaps, imbibition in the wall of the tracheole itself."

This activity continues until the hooks break through. The animal immediately crawls out, enlarging the opening by means of bodily pressure. No swallowing of the amniotic fluid or of air was observed, as described by SIKES and WIGGLESWORTH (1930) for other forms. The above description is from wet mounts.

In studying intact eggs on a slab of food, and eggs suspended from the surface film of a drop of Ringer's fluid, it was seen that the larvae emerge by breaking through a flap, which is dorsally hinged, at the anterior end of the chorion. The chorion in this region is more translucent than elsewhere. The translucent area is demarcated posteriorly by an irregular boundary which passes dorso-ventrally around the egg about one-third of the distance from the anterior end on the dorsal side, just in front of the bases of the two filaments, and about one-fifth from the anterior end on the ventral side. E. H. STRASBURGER (1935) figures a difference in the architecture of the chorion in this region. To effect a final exit from the surrounding membranes, the larva must be able to brace itself on some solid surface outside the egg, normally the food surface. Thus, in those cases where the eggs were suspended in the Ringer's fluid, though the larvae broke through the membranes, they could not free themselves completely.

Histomorphology

An extremely thin epidermal cuticle is already present at this stage, and it has undergone regional differentiation into thorn-shaped spines. The spines vary in shape and size. On the head they are located around the edge of the mouth and lateral lips. Here they are long and thin. Posteriorly they are located in rows on either side of the intersegmental junctures (with the exception of the first thoracic segment). On the ventral and lateral portions of the cuticle, the spines are short and have broad bases (figs. 1 and 8: CS). In wild-type larvae the tips appear to have a deposition of dark brown pigment. On the dorsum, they are very delicate, colorless, and are generally scattered over the surface.

No distinction can be made between an epicuticle and endocuticle (see WIGGLESWORTH 1933). There is only a single layer, which does not stain with the dyes used.

The hypodermis consists of a single layer of flattened cells with elliptical nuclei (fig. 6 H). The cells extend into the bases of the broader spines, but no specialized trichogens or similar structures are evident. A basement membrane, if present at this stage, cannot be distinguished. Occasional loose aggregates of spherical or amoeboid cells lie in the body cavity in close juxtaposition with the hypodermis.

The imaginal discs are represented at this stage by all three pairs of

ventral thoracic discs and by the dorsal meso- and metathoracic discs (figs. 1, 6, 9, and 10: PT, MT, METD, MET). The description of these in the newly hatched larva, as given by AUERBACH (1936), corresponds exactly to our observations. In addition, we observed the anlage of the genital disc (fig. 5 GD). This arises as a proliferation of cells in the ventral region of the hypodermis just anterior to the anus. The disc has already begun to invaginate. In cross section it is broad and flat; in longitudinal section it appears as a tongue of tissue pointing anteriorly.

Though the frontal sac has invaginated by 12 hours (at 25°C) the differentiation of its posterior portion into the discs of the cephalic complex has not yet taken place at 22 hours.

The musculature of the embryo is already functional at 22 hours. In section the musculature of the pharynx is very characteristic (figs. 1 and 9: PHM, PHP). The body musculature (figs. 1 and 9: BM) is characterized by an intricate system of fibres running longitudinally and another set running obliquely and crisscrossing. No attempt was made to study these in detail. SNODGRASS (1924), figures their arrangement in the apple maggot. HERTWECK (1931) figures the system in the mature larva of *D. melanogaster*. The arrangement in both forms is very similar.

The presence of smooth muscle fibres in the gut and the bases of the Malpighian tubules is indicated by the active peristalisis of these regions. These fibres are extremely difficult to find in section. EASTHAM (1925) figures them in the hindgut and Malpighian tubules of third instar larvae of *Drosophila funebris*.

A description of the development of the nervous system in the embryo has been given by POULSON (1937b). HERTWECK (1931) gives a description of this system in the larva and adult. Our observations (figs. 1, 7 and 8) for the late egg stages agree fully with POULSON'S.

The alimentary tract is fully differentiated by 20 hours. A description of its anatomy is given by M. STRASBURGER (1932).

Histologically, the various regions of the gut differ in the shape and size of the cells composing the single-layered epithelium (figs. 7 and 8). The nuclei are all similar in appearance, though not in size. They are spherical, and, with the exception of the salivary gland nuclei, among the largest in the animal at this stage.

In the oesophagus (fig. 7 O) and the hindgut (fig. 8 HG) the cells are triangular in section, with their apices directed toward the lumen of the gut. The cells of the oesophagus are the smallest in the alimentary tract.

In the anterior portion of the midgut and in the four enteric caecae the cells are subcolumnar. These cells are the largest of the alimentary tract (with the exception of the salivary gland cells). They undergo a gradual decrease in size and change of shape from the anterior to the posterior

portions of the midgut (fig. 8: AG, PG). In the middle and posterior portions they vary from subcolumnar to pavement. The entire epithelial layer of the midgut is characterized by a brush-like inner border, the "Stäbchensaum." The cytoplasm, with the fixatives used, is eosinophile and vacuolated. No studies were made of mitochondria, Golgi, or other formed bodies.

The salivary glands are differentiated into a duct portion and a glandular portion, as POULSON (1937b) has observed. The glandular portion consists of a single cell-layer of large pyramidal cells (fig. 9 S). The nuclei of these cells already have a distinctive appearance. They are the largest in the animal, and contain spiral chromatin threads which stain more heavily with gentian violet than do those of other tissues. As POULSON observes, they have not as yet acquired the characteristics associated with them in older larvae. The cytoplasm stains very lightly, and contains a large vacuole situated in the apex of the cell, between the nucleus and the lumen of the gland.

The Malpighian tubules, as POULSON states, are already pigmented. The distribution of the pigment has been described in a previous section. The calcium carbonate contents of the tubules do not appear in fixed material, probably because of their solution by the acetic acid of the fixatives.

With the exception of the atria and the distal portions of the anterior tubules, the histological structure of the Malpighian tubules is uniform. The single layered epithelium is composed of pavement cells (figs. 5 and 7: MP) whose inner surfaces are rounded and carry long filaments, the so-called "Bürstensaum," which appear to be absent from the distal portions of the anterior tubules. In the atria, they are very long, extending into the gut lumen. In fixed sections, the individual fibres of the *Bürstensaum* lose their identity. The cytoplasm of the cells, like that of the salivary gland cells, stains very lightly, and appears highly vacuolated.

The tracheal system is fully developed by 22 hours. The tracheal cuticle is formed, and has been thrown into spiral folds, the "taenidia." The tracheal epithelium consists of pavement cells with small nuclei.

The future two anterior spiracles (fig. 10 AS) are indicated by a posteriorly pointing cord of cells attached to the hypodermis of each dorsolateral wall of the prothoracic segment. The posterior spiracles are fully developed. They are carried on a papilla on the dorsal aspect of the last abdominal segment. The spiracular vestibules are thickly lined with yellow bristles (fig. 5 SV). The spiracular orifices are surrounded by a circle of long spines.

Due to the longitudinal compression of the larva in the egg membranes, the anterior portions of the great lateral tracheal trunks form U-loops in the region of the thoracic segments. This portion of the trunks seems to

be particularly flexible, since in the alternate lengthening and shortening of the larval body during locomotion the trunks flex and unflex in this region.

Gas appears in the tracheal system about two to two and one-half hours before hatching. This has been described in detail in a previous section. The tracheal system of the larva has been described by RUHLE (1932), and HASKINS and ENZMANN (1936).

The heart, as POULSON (1937b) has observed, is actively beating at this stage. It is extremely difficult to find in sections. No detailed study of it was made.

The fat bodies are most conspicuous in the head region (fig. 10 FB). There are also small patches in the dorsal region of the abdomen. The deposition of fat in the cytoplasm is as yet slight. In the head region, the fat bodies surround the salivary glands and the distal portions of the enteric caecae. They also lie lateral to the pharyngeal dilator muscles.

FIGURE 7.—Cross section through the first abdominal segment of a wild-type egg 22 ± 1 hours old. 423×.

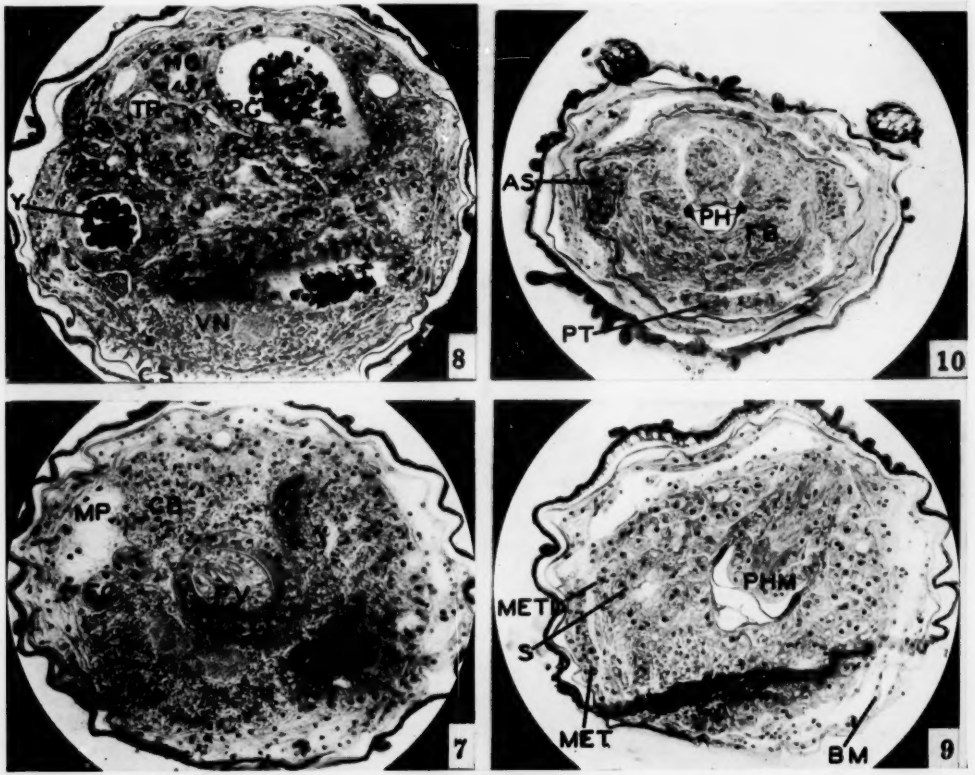
FIGURE 8.—Cross section through the inter-segmental membrane between the third and fourth abdominal segments of the same egg as in figure 6. 423×.

FIGURE 9.—Cross section through the metathoracic segment of the same egg as in figure 7. 423×.

FIGURE 10. Cross section through the prothoracic segment of the same egg as in figure 6. 423×.

ABBREVIATIONS

AG	anterior portion of the midgut
AS	anterior spiracles
BM	body musculature
C	chorion
CB	cerebral ganglion
CS	cuticular spines
CU	cuticle
EC	enteric caecae
FB	fat body
GD	genital disc
GL	great lateral tracheal trunks
H	hypodermis
HG	hindgut
LP	lateral process of the vertical plate of the mouth armature
LT	segmental branches of the tracheae
MET	ventral metathoracic imaginal disc
METD	dorsal metathoracic imaginal disc
MG	midgut
MH	mouth hook
MP	Malpighian tubule
MT	ventral mesothoracic imaginal disc
MZ	medianzahn of the mouth armature
O	oesophagus
PG	posterior portion of the midgut
PH	pharynx
PHM	pharyngeal dilator muscles
PHP	pharyngeal protractor muscles
PM	posterior Malpighian tubules
PT	ventral prothoracic imaginal discs
PV	proventriculus
S	salivary glands
SD	common duct portion of the salivary glands
SV	posterior spiracular vestibules
T1	first thoracic segment
TR	trachea
VM	vitelline membrane
VN	ventral ganglion
VNC	cell body portion of the ventral ganglion
VNF	fibrous portion of the ventral ganglion
Y	yolk
1, 2, 3,	first, second, and third thoracic segments
I, II, III, IV, V, VI, VII,	seven abdominal segments



FIGURES 7–10

THE LETHAL-BEARING EMBRYOS

The eggs which have failed to hatch by 24 hours after oviposition, and thus presumably are the hemizygous lethal type, can be seen, with a magnification of 20×, to contain larvae whose yellow mouth armature and posterior Malpighian tubules are easily visible through the translucent chorion. Such eggs were examined in wet mounts at magnifications of 150× and 660× and in dissections.

The mutant larva cannot be distinguished from the normal animal of comparable age (22–24 hours) as regards its organology. As has been stated, the chief differences between mutant and normal are in the condition of the tracheae with respect to the distribution of gas in them (figs. 3: TR, GL; 4) and in the hatching activities.

Whereas in the normal larva, the tracheal system is completely filled with gas at 22 hours (fig. 2: GL, LT), the great lateral trunks of the mutant almost never contain gas. Gas may at times apppear in the smaller branches that pass ventrally. Among more than fifty mutant eggs that were observed, only two showed any gas in the great lateral trunks, and in these two cases the gas filled about two-thirds of the trunks and extended posteriorly only as far as the penultimate abdominal segment. It should be emphasized that *structurally* the tracheal system appears to be normal.

The feebleness of the motions of the mutant within the vitelline membrane is very striking. The bodily contractions are weaker and not as frequent as in the normal. The mouth hooks rarely enter the micropyle, and when they do, generally fail to pull the membrane back on retraction. The exploratory movements of the hooks are ineffectual. Thus when the tips of the hooks come in contact with the vitelline membrane, the hooks usually buckle at their posterior joint with the mentum, so that their dorsal surfaces rather than their tips are in contact with the enclosing membrane. Of the fifty or so mutant eggs that were observed, none was seen to hatch.

These observations support our conclusions that the mutants do not hatch. It might be argued that the technique of dechorionating eggs and studying them in Ringer's solution introduced factors which prevented hatching. However, normal eggs did hatch under these conditions.

The heavily charged condition of the posterior Malpighian tubules of the 24 hour old mutant is another striking feature (figs. 3 and 4: PM). It is this factor which makes them visible through the chorion. The comparison is made with reference to the normal embryo at about *20–22 hours* of age, since most normals have hatched by 24 hours. The difference between the mutant and normal embryos may thus be due to the fact that in the former the accumulation of concretions has been in progress for two hours more than in the latter at the time the comparison was made.

No conclusion on this point is possible from purely empirical comparisons with the degree of accumulation in *first instar normal larvae* shortly after they have hatched, and it is not known whether there is any significant difference between the mutant and normal in this respect. The subsequent condition of both the anterior and posterior pairs of tubules in mutants that are more than 24 hours old, shows that accumulation of concretions continues throughout the life of the animal. This will be described below.

Histomorphology of the mutant larva

In sectioned material, except for one difference, the late mutant embryo is indistinguishable from the normal of comparable age (22 hours). This has been determined from a study of over 200 sectioned mutants. The difference is in the pigmentation of the pharyngeal armature, this being gray-black in the non-yellow normals and yellow in the mutant; and the depth of pigmentation of the ventral cuticular spines, which are more deeply pigmented in the non-yellow normals than in the mutants. As has been pointed out previously, the pigment difference is a phenotypic expression of the absence of the locus for yellow, and gentically yellow normal embryos of comparable age look like the mutant in this respect.

THE TIME OF DEATH OF THE MUTANTS

During the course of fixing eggs for histological study it had been noticed that nearly all those that had remained on the original food slab 48 hours after they had been laid were dead. This was shown by their brown color, due to decomposition, by their lack of firmness when pressed, by the opacity of the eggs on direct examination under magnifications of 150× and 660×, and by the absence of response of the embryos to prodding with a needle. The data for all the experiments that follow are listed in table 2. Of 49 eggs that were about 48 hours old when examined, only three were alive (exps. IIA; VI 6). All of eleven eggs examined at 36 hours were alive (exp. III). These observations seemed to place the time of death fairly close to 48 hours.

In order to determine the time more accurately, mutant eggs were dechorionated 24 to 36 hours after they had been laid and examined in wet mounts. Many of these embryos lived much longer than those left intact on the food slab. Of five eggs dechorionated when 24 hours old, one was still active at 72 hours (exp. IV 2). This was an exceptional animal in that when first observed it had the last two or three abdominal segments reflected over the dorsum. After it became free of its vitelline membrane, either by hatching which had not been observed, or through pressure of the overlying coverslip on the membrane, it was obviously longer and larger than the average larva at hatching time.

TABLE 2

Longevity of the mutants under differing external conditions.

EXP. NO.	AGE OF EGGS AT BEGINNING OF OBSERVATIONS	NO. OF EGGS	TREATMENT OF EGGS	CHARACTER OF EXTERNAL MEDIUM	ELAPSED HOURS AT TIME OF OBSERVATION	NO. EGGS ALIVE AT ELAPSED TIME	TEMP. °C
	HOURS						
II A	46±4	10	dechorionated	Ringer's solution	46±4	3	23–24
					53	2	
					63	1	
					77	1	
					80	0	
II B	55±4	8	dechorionated		55±4	0	23–24
III	36±1	11	dechorionated	Ringer's solution	36±1	11	23–24
					50	1	
					63	1	
IV 2	24±1	5	dechorionated	Ringer's solution	24±1	5	26±
					51	1	
					72	1	
V 1	25.5±1	10	dechorionated. Two larvae broken out of both membranes	2% agar dissolved in distilled water	25.5±1	10	25±½
					43	9	
					66	5	
					78	0	
V 2	24±1	9	dechorionated	"	24±1	9	25–26
					43	7	
					66	3	
					78	0	
VI 4	24±1	10	membranes intact. Washed in three washes of distilled water	"	24±1	10	25–26
					47	5	
					53·5	1	
VI 9	24±1	8	dechorionated	"	24±1	8	25–26
					48	7	
					72	3	
					80	0	
IV 1	24±1	6	4 dechorionated, 2 intact	2% agar in 20% molasses	24±1	6	26.5±
					48	0	
VI 7	28±1	10	dechorionated	"	28±1	10	25–26
					31.5	10	
					48	0	
VI 8	28±1	10	membranes intact. Eggs washed	"	28±1	10	25–26
					48	0	
V 3	24±1	10	8 dechorionated. 2 eclosed fr. both membranes	Original food slab. Surface yeast and larvae removed	24±1	10	25–26
					48	1	
					60	0	
VI 6	48	39	Examined from the original food slab		48	0	25–26

Of eleven embryos that were dechorionated when 36 hours old, one was still moving feebly at 63 hours (exp. III).

In two experiments the eggs were placed on agar slabs (2 percent agar dissolved in distilled water) on paper milk-bottle caps which had previously been divided into numbered squares with a pencil. Eighteen eggs, 24–25 hours old, were dechorionated and treated in this manner, (exps. V 1 and VI 9). In exp. V 1, five out of ten eggs were still alive at 66 hours. In exp. VI 9, of eight eggs, three were still alive at 72 hours. It should be noted that normal larvae enter the second instar about 24 hours after hatching from the egg, and the third instar about 24 hours later (AUERBACH 1936). During this period they undergo two moults and a tremendous increase in size.

With the possible exclusion of the exceptional animal mentioned above, no growth or apparent further differentiation took place in the mutants. On the contrary, they decreased to about three-fourths their original size by 48 hours.

The possible cause of the earlier death of the mutants on the original food slab

An attempt was made to determine the significance of the difference in longevity between the eggs remaining on the original food slab, and those that were placed on other media.

Several possible causes of the difference can be formulated: 1) the treated eggs had their chorions removed; 2) the eggs remaining on the food slab were subjected to a hypertonic medium due to the presence of metabolic products from the growing yeast and the first instar normal larvae, and from the high molasses concentration (20 percent); 3) the metabolic products might diffuse through the egg membranes and have a toxic effect on the untreated eggs.

The mutant eggs used in the experiments to be described were 24–28 hours old at the beginning of the observations.

The role of the egg membranes. Fourteen eggs were dechorionated and placed on unyeasted slabs of two percent agar dissolved in 20 per cent molasses. At 31 hours the eggs were getting soft (exps. IV 1 and VI 7). At 48 hours all were completely dehydrated.

In experiments IV 1 and VI 8, twelve eggs, left intact, were washed in four changes of distilled water to free them from yeast and put on unyeasted agar-molasses slabs. The washing did not effect its purpose since the eggs acted as centers of yeast growth. At 48 hours all the eggs were dead and soft; some loss of amniotic fluid and some shrinkage of the embryos had taken place, though this was not as marked as in the eggs with the chorions removed. Apparently the chorion is less permeable to water than the vitelline membrane.

In contrast to these results should be kept in mind the increased length

of life of those eggs that were dechorionated and placed either in Ringer's fluid or on agar slabs as described in the previous section.

To further test the effect of the chorion, ten eggs (exp. VI 4), not dechorionated, were washed in three changes of distilled water to free them of yeast, and placed on an agar slab (two percent agar dissolved in distilled water). At 47 hours, five were alive, and at 53.5 hours, one was still alive. The numbers, though not conclusive, are indicative when it is recalled that in exp. VI 6 all of 39 eggs examined that had remained on the original food slab were dead and soft at 48 hours.

In exp. V 2, nine mutant larvae were freed from both membranes and placed on agar-water slabs. Three were still alive at 66 hours. None of the larvae showed any further growth or differentiation. All remained at the exact spot on the agar on which they had been placed. They were flaccid and collapsed at the beginning of the observations, and quite turgid at the end.

From these experiments we conclude that within the limits of sensitivity of the methods used, the presence or absence of the membranes does not influence the length of time the mutants live after they have become differentiated as larvae.

The effect of the external medium. The experiments described in the preceding section indicate that some factor or factors in the external medium affect the longevity of the mutant.

To test this further, in experiment V 3 ten mutant eggs were dechorionated to make observation easier and placed on a slab of food from which the normal larvae and the surface growth of yeast had been removed. Two of the larvae were accidentally broken out of the vitelline membranes. All but one were dehydrated and dead at 48 hours. One remained alive within the vitelline membrane. This animal was exceptional for a mutant in the degree of vigor of its movements within the membrane. The right great tracheal trunk was filled with gas up to the posterior spiracular chamber, and the fluid column in the left great tracheal trunk was broken up by bubbles of gas. In the hope that it might develop further, it was freed from the vitelline membrane and placed on fresh food. However, it showed no coordinated crawling movements, and no feeding movements. It died within 12 hours after eclosion.

As controls we have the experiments mentioned in the two previous sections. In experiments III and IV 2 there was a marked increase in longevity when the eggs that had been dechorionated were placed in Ringer's fluid. In experiment V 1 and VI 9 there was a marked increase in longevity when the dechorionated eggs were placed on agar-water slabs. There was likewise an increase in longevity in the case of the larvae that were eclosed from both membranes and placed on agar slabs (exp. V 2). In

experiment VI 4, the intact eggs that were washed free of yeast and placed on agar slabs showed a significant increase in longevity.

On the other hand, in all the cases where the eggs *whether dechorionated or not*, were placed on *unyeasted* slabs of two percent agar dissolved in 20 percent molasses, (exps. IV 1, VI 7, VI 8) they were dead and dehydrated by 48 hours.

All the media on which death occurs early have one thing in common: they are hypertonic with respect to the animal. This would indicate that the early death of the mutants observed in these cases is correlated with the *dehydrating* effect of the external medium. Normal larvae, of course, resist dehydration perfectly on the usual food media.

THE APPEARANCE OF THE MUTANT LARVA SHORTLY BEFORE DEATH

The following account is based on the long-lived mutants in dechorionated eggs that were placed on slabs of agar dissolved in distilled water.

Such eggs contain larvae that have undergone a marked shrinkage. This shrinkage corresponds with an increase in the surrounding amniotic fluid, so that the original turgor of the egg is retained. The amniotic fluid becomes filled with many globules which are in active Brownian movement. Droplets of liquid often condense on the surface of the vitelline membrane.

The larval cuticle is extremely wrinkled, probably as a result of shrinkage of the animal. Characteristically, those animals that are eclosed from the vitelline membrane also, do not exhibit this shrinkage in external body form, but the internal organs are markedly decreased in size. The difference may be due to the fact that the latter animals are no longer surrounded by the amniotic fluid which, in the former, must be gradually getting more hypertonic with the accumulation of degradation products from the enclosed larva. In the case of the animal that is eclosed from both membranes these products have the possibility of diffusing into the external medium and becoming diluted. In fact, the larvae that are eclosed from both membranes, become more turgid after death, presumably as a result of endosmosis.

The distal portions of the anterior pair of Malpighian tubules, as well as the entire posterior tubules, become heavily charged with calcium carbonate concretions. This corresponds to their condition in normal first instar larvae some time after the latter have hatched.

With the onset of death, the outlines of the internal organs gradually lose their sharpness. The animal takes on a brownish tone with the progress of decomposition.

GENERAL CONCLUSIONS

The possible significance of the observed difference between the mutant and normal larvae. With the histological techniques employed we have not

been able to demonstrate any structural difference between mutant and normal.

The chief differences distinguishing the mutant from the normal are: (1) the sluggishness and apparent debility of the mutant; (2) its failure to hatch; (3) the atypical distribution of gas in the tracheae; and (4) the inability of the mutant to withstand dehydration.

Point (2) may be a consequence of point (1). Points (3) and (4) may have the same causal background if we assume that the cuticle of the mutant is partially permeable to water, while that of the normal is wholly impermeable.

SIKES and WIGGLESWORTH (1930) believe that the first appearance of gas in the tracheae must be correlated with a change in permeability of the tracheal lining, so that only gases will diffuse through it. If, in the mutant, the cuticle remains partially permeable, the complete displacement of the tracheal fluid would be prevented and gas might be expected to appear only in those branches which are closest to the regions of highest osmotic pressure, that is, the muscle tissues.

The assumption of partial permeability would also explain the dehydration of the mutant. Thus, the observation that the mutants that are eclosed from both membranes retain a smooth external contour when placed on a hypotonic medium, would indicate the possibility of endosmosis through the cuticle. This may be the only means of water intake, since the mutant does not feed, and therefore fails to imbibe water.

But it is possible to discard the assumption of a difference in permeability and still have a consistent picture. All the observations described are explainable, if it is assumed that the debility of the animal is associated with a lower metabolic level. Thus, keeping in mind the correlation between the *degree of activity* of the embryo and the time of onset of gas in the tracheal system as formulated by SIKES and WIGGLESWORTH (1930), it is possible that the erratic character of the distribution of gas in the tracheae of the mutants is correlated with their lower metabolic level and consequent lower osmotic pressure in comparison with the normal. Their general debility might also be responsible for the failure to hatch, lack of coordinated crawling movements when eclosed from the egg membranes, and absence of feeding movements.

The differences in appearance between the mutants that are eclosed from both egg membranes and show no subsequent shrinkage and those that are not eclosed and do show shrinkage can be explained on the assumption that the former do imbibe water passively through the mouth and anal orifice from the surrounding hypotonic medium, that is, the slab of agar dissolved in distilled water. Also, the collapse of the mutants on eclosion, as previously described, can be attributed to their weak musculature which can not counteract the distorting forces of gravity and

surface tension. Thus, in contrast, mutants immersed in Ringer's fluid or tap-water, being supported by the surrounding fluid, retain a normal contour. (In this connection, a weakly sclerotized cuticle would give the same results.)

A decision can not be made, therefore, on the basis of the data presented, as to whether or not there is a difference between the mutant larva and the normal in the condition of the cuticle.

The condition of the Malpighian tubules. The larvae of many Diptera are known to contain calcium carbonate in the Malpighian tubules. This is true of larvae which are phytophagous, saprophagous, or parasitic (WIGGLESWORTH 1934). As a rule the calcium carbonate is stored in the tubules in the form of small granules in a thick suspension, or as large calcospherites both in the fat bodies and tubules (KEILIN 1921). According to KEILIN, "The excretion of calcium carbonate in Dipterous larvae is comparable with calcareous excretion as observed in other organisms . . . where this product of excretion is supposed to be derived from the neutralization of the carbon dioxide of respiration. This explanation has not yet been proved experimentally."

WIGGLESWORTH (1934), however, points out that "the amount of carbon dioxide bound in this way must form but a fraction of what the insect produces. Perhaps these carbonates simply provide a mechanism for getting rid of excess alkali; it is at least noteworthy that they occur chiefly in saprophagous and phytophagous larvae of Diptera . . . whose food might be expected to contain an excess of fixed base. Their urine must therefore be alkaline, and in the presence of the carbon dioxide of the tissues, if calcium or magnesium ions are present in quantity, carbonates will tend to precipitate."

In the mutants that survive beyond 48 hours, the accumulation of concretions in the Malpighian tubules corresponds in location and quantity to that of the normal first instar larva. Since the mutant does not feed, its only source of calcium is the supply originally accumulated in the egg during oögenesis. If, as WIGGLESWORTH supposes, the deposition of calcium carbonate is an expression of the alkaline condition of the body fluids, then in *Drosophila melanogaster* the high pH is an inherent adaptation to the phytophagous feeding habits of the species.

DISCUSSION

Deficiencies and lethality. POULSON (1937a), in a comparative study of the lethal effects of deficiencies of different lengths in the X chromosome of *D. melanogaster* found that the longer the missing piece was, the earlier in ontogeny did the lethal disturbance occur.

DEMEREC and HOOVER (1936) made a comparative study of three termi-

nal deficiencies of the left end of the X chromosome in *D. melanogaster*. They found that the shortest of the three, def. 260–5, which does not include the yellow locus, is neither cell-lethal nor zygote-lethal. Its only phenotypic expression is a partial sterlity in homozygous females. Def. 260-2 (studied in this paper), which includes at least the loci chlorotic, lethal J-1, yellow, and achaete, is not cell-lethal but is zygote-lethal. Def. 260-1, which covers the same loci as def. 260-2 and also includes the locus for scute, is both cell-lethal and zygote-lethal.

The impression that one gathers from the above cases is that there is a direct correlation between the size of a deficiency and the magnitude of its effect on development. In general, this is probably a correct formulation, but as MULLER (1935) puts it: "A lethal effect (in the case of a deficiency) is not so inevitable as it might seem at first sight, for the organism . . . has available to it many processes of regulation and compensation that may be called into action in cases of disturbances of the normal equilibrium, including among the latter even changes that are genetic in origin. Nevertheless, it is not to be expected that the organism could withstand the effects of the removal of many genes at once, just as it could not usually survive the removal of many organs at once."

Aside from the assumption that the magnitude of a deficiency, if it involves a fairly large number of loci, must make it lethal, it is conceivable that a deficiency involving very few, or even only one, loci may be lethal. Putting it another way, different loci may have different "vitality values."

The question then arises as to whether, in the present instance, the lethality may be ascribed more to the absence of a specific locus, than to the fact that a number of loci are missing. Discussion on this point must remain purely speculative at the present time because of the scantiness of data, but several observations of interest bearing on this problem should be mentioned.

MULLER (1935) studied a *viable* deficiency which probably involved only the loci yellow and achaete. DEMEREC and HOOVER (1936) believe that the def. 260-5 studied by them extends up to the locus for lethal J-1, which is to the left of yellow, but does not include it. They base this conclusion upon the assumption *that lethal J-1 is hypomorphic*, and its deletion would therefore give a lethal genotype. Def. 260-2, studied in the present paper, is presumably terminal, and extends up to, but does not include, the locus for scute. This deficiency may therefore differ from MULLER'S in that it includes loci to the left of yellow, among which would be lethal J-1. The lethality of def. 260-2 may therefore be ascribed to the absence of the locus lethal J-1.

The mode of action of the lethal deficiency 260-2. As stated in the introduction, the lethal genotypes investigated to date have been classified

as to their mode of action into two categories (DAVID 1936). This classification holds, of course, for the level at which investigations have been made heretofore, namely, in terms of the morphological effects; but in all cases the effect must eventually be traced back to some deviation from the normal biochemical organization.

The test used to differentiate the two categories has been to culture tissues and organs in vitro (EPHRUSSI 1935; DAVID 1936). If a tissue is inherently pathological, it should not survive when cultured. (It is, of course, conceivable that a tissue might prosper in cultures and yet have a specific lethal effect because it acts as a source of toxins.) Methods of in vitro cultures of insect tissues have not been devised yet, but in Drosophila the technique of transplantation has been employed by HADORN (1937).

The cases studied to date can not be exclusively consigned to one or the other of the two categories. POULSON'S (1937a) cases of half-X and nullo-X probably fall in the first category, as do the cases of cell-lethals (DEMEREC 1934a, b; 1935, 1936; EPHRUSSI 1934; STERN 1935). POULSON'S cases may be looked upon as cell-lethals, in a broad sense, since the cleavage nuclei are probably autonomous to a large degree. The lethal anemia caused by the "black-eyed white" gene in mice (LITTLE 1915; DETLEFSEN 1923; DE ABERLE 1927; GOWEN and GAY 1932) may be included in the first category. Death here is traced to a deficiency of the blood cells. Whether "brachyury" in the house mouse (CHESLEY 1935; EPHRUSSI 1935) should be included in the first or second category is still a point at issue.

In the second category may be included the "Creeper" fowl (LANDAUER 1932, 1935; DAVID 1936), and the "lethal-giant" larva (HADORN 1937) in *D. melanogaster*. Lethal-7 in *D. melanogaster* (STARK 1918, 1919, 1937; MORGAN, BRIDGES and STURTEVANT 1925) should properly be included in this category. The last named authors point out that from STARK'S data it is impossible to decide unequivocally between her conclusions that (1) the prime cause of lethality is the deleterious effect resulting from the tumors found in lethal-7 larvae or (2) that the lethal effect of the gene is expressed in the already weakened condition of the larva, the tumors being a secondary manifestation. That the latter may be the case gains support from the fact that a lethal-7 stock which was in the laboratory for several years no longer produced larvae with tumors. That the lethal was still present and acting was borne out by the aberrant sex-ratio, and by suitable crosses with closely linked marker genes. The tumors reappeared in the stock after outcrossing.

The mutant studied in this paper does not fall into either of the two categories, since its histomorphology appears to be normal, though the aberrant distribution of gas in the tracheae may be considered a morpho-

logical as well as physiological abnormality. With this exception it most closely approximates the second category of cases. Apparently the complex set of events leading to final organization of a larva from the fertilized egg proceeds undisturbed in these animals. The disturbance, metabolic in character, first becomes apparent when the animal is about to enter the next stage in its ontogeny, that of a free-living larva.

That this particular period in the ontogeny of Drosophila may be a critical one is indicated from observations of other workers on the time of action of lethals. LI (1927) records the death of Plexate animals, homozygous for a small deficiency in the second chromosome, in the late egg stage. The larvae are fully formed, as evidenced by the presence of the mouth armature and the tracheal system, but fail to hatch. POULSON (1937a) and BREHME (1937) describe somewhat similar cases for scute-8 and ClB respectively.

Similar evidence can be adduced from other forms, namely the silkworm, *Bombyx mori* (NISHIKAWA 1931; TANAKA 1925), and the meal-moth *Ephestia kühniella* (SCHWARTZ 1937). In these cases also lethal-bearing larvae are fully formed in the egg, but fail to hatch. SCHWARTZ (1937) further finds that in sectioned material of the Ephestia lethal there are no observable differences between the lethal and normal animals.

In our case the deficiency thus is shown to so modify the metabolic conditions in the late embryo as to produce death without any apparent morphological alterations. The other lethals just cited probably operate through similar channels. Lethal action in these cases is not an extension of a deleterious morphogenetic effect.

ACKNOWLEDGMENTS

The author wishes to express his thanks to DR. M. DEMEREC for so kindly making available the stocks of flies, and to MR. JACK GODRICH for help in preparing the illustrations.

This investigation was undertaken at the suggestion of PROFESSOR L. C. DUNN. It is a pleasure to acknowledge the author's indebtedness to PROFESSOR DUNN for his sustained interest and aid during the course of the work.

SUMMARY

1. In *Drosophila melanogaster*, the genotype characterized by a terminal deficiency involving at least four known loci, and possibly five, at the left end of the X chromosome, and the absence of eight bands from the salivary chromosome, is zygote-lethal when hemizygous.

2. Male zygotes carrying the deficient chromosome develop to fully formed larvae but fail to hatch from the egg. Under the proper conditions,

they may live without further growth or differentiation for a period of time during which normal larvae will have entered the third instar.

3. Examination of living mutants disclosed that their chief characteristics are; (a) their relative inactivity and apparent debility; (b) the failure of the tracheal system to completely fill with gas; (c) their failure to hatch; and (d) their inability to withstand dehydration. Mutants that are eclosed from the egg membranes exhibit no coordinated crawling or feeding movements.

4. Histological examination of mutants and normals of comparable age indicates that the histomorphology of the mutants is normal.

5. Two hypotheses are advanced as generalizations of the observations. The first states that all the characteristics of the mutant can be explained on the assumption that it has a low rate of metabolism. The second hypothesis, not necessarily alternative to the first, states that if it be assumed that the larval cuticle of the mutant remains semi-permeable to water, this would account for the condition of the tracheae and the inability of the animal to withstand dehydration.

6. The pigmentation of the mouth armature and the cuticular spines in the mutant is "yellow-type," supporting MULLER's classification of the yellow locus as hypomorphic.

7. A description is given of the histomorphology of the normal larva about two hours before hatching time, of the manner of the spread of gas in the tracheal system, and of the events leading to hatching.

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HAIRY WING—A DUPLICATION IN DROSOPHILA MELANOGASTER

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Received November 23, 1938

A LARGE proportion of the X chromosome deficiencies maintained in stock at this laboratory are balanced over In(1)dl-49, *y Hw*. When such deficiencies were examined cytologically, it was frequently noticed, especially when the chromosomes were unpaired, that there was some departure from the normal salivary gland chromosome banding of the tip region of the X carrying *Hw*. Since such material was heterozygous for *Hw*, which is known to be genetically close to this free or left end of X, it seemed logical to investigate whether the presence of *Hw* might account for the abnormality observed. Evidence obtained later from duplications for the left end of X gave further support to this theory. Flies carrying duplications obtained from the translocation T(1·4)*w* 258-18 (DEMEREC and SLIZYNSKA 1937) were phenotypically Hairy wing. This finding suggested that the expression of Hairy wing was due in itself to a duplication for some portion of the X chromosome close to the tip and consequently experiments were planned to determine the presence of such a duplication cytologically and to test its behavior genetically.

Following are descriptions of mutants and aberrations which were used in this work. These are adapted from descriptions prepared by C. B. BRIDGES for Drosophila Information Service, No. 9.

The mutant character Hairy wing (*Hw*) was found by C. B. BRIDGES in 1923 as a spontaneous mutation in a *y cv t f* stock. It has not been possible to separate it by crossing over from *y*, placing its locus, therefore, at 0.0±. Males carrying *Hw* show extra bristles along the wing veins, on the head, affecting especially the occipitals, and on the thorax. Extra hairs also occur on the back of the head and on the pleurae. Females heterozygous for *Hw* show some extra bristles especially on veins; females homozygous for *Hw* exhibit a more extreme character than the males. They are not lethal, but their viability is reduced to 80 percent. The rank accorded the character as based on viability and ease of classification is 1.

The mutant achaete (*ac*) was found in 1916 by WEINSTEIN (1918) as a spontaneous change in the yellow stock. DUBININ referred to it as sc^{11}. It has not been possible to separate it from *y*, but cytological studies place its locus between *y* and *sc*, thus 0.0+. On homozygous flies the posterior dorsocentrals are missing, while the anterior dorsocentrals are rarely affected; hairs are usually fewer in the neighborhood of the posterior dorso-

centrals; intraocular hairs are invariably fewer and typically absent; eyes are partly devoid of hairs.

Deficiency Df(1) 260-2 was found among offspring of an X-rayed male by DEMEREC in 1933 (DEMEREC and HOOVER 1936). Genetically it included *y* and *ac* but not *sc*. It is lethal and cell-lethal in the homozygous condition. Salivary chromosome analysis shows a piece missing from the tip up to and including 1 B1·2. Salivary chromosome bands are numbered according to BRIDGES' (1938) second map.

Duplication Dp(1; f) 118 was found among offspring of an X-rayed male by DOBZHANSKY (1932). Genetically it covers *y sc svr* and also *bb* but not *br*. Metaphase plates show a free fragment about one quarter length of the X chromosome.

CYTOLOGICAL RESULTS

It has been previously mentioned that during the cytological study of certain deficiencies and inversions a slight disturbance in normal banding was noticed near the tip of the X chromosomes. Since these X chromosomes were genetically known to carry *Hw*, the possibility that *Hw* was responsible for this abnormality was investigated. Selected material was used in which in a heterozygote the chromosome carrying *Hw* could be cytologically distinguished from the wild type or normal homologue. Inversion *ct* 268-13 *y Hw/ct v dy g f* in which *Hw* was carried in the same chromosome as a tandem inversion involving practically the entire chromosome (HOOVER 1937) was useful; the presence of the long inversion results in a fairly high proportion of unsynapsed chromosomes and the inverted order of the banding in one of the haploids makes the *Hw* chromosome easily detectable. Deficiency *N* 264-30/dl-49, *y Hw* was used because a large 18-band deficiency, 3 A 5 to 3 C 8 inclusive, made it possible to distinguish the strand carrying this deficiency from the one carrying *Hw*. In the *w* 258-18/dl-49, *y Hw* material one chromosome carries a translocation with one break in the X between 3 C 3 and 5 and the other in the chromocenter of the fourth chromosome. The X tip attached to the fourth chromosome is frequently unsynapsed due to the chromocentral attraction, and the unpaired *Hw* chromosome is also distinguishable by the dl-49 inversion which it carries.

In such material, the haploid strand known to carry *Hw* contrasts with its homologue in the heavier appearance of the 1 B 1·2 band. Careful analysis confirmed the fact suggested by a preliminary study of these chromosomes in the paired diploid condition, namely, that this band was duplicated. Although this band has been figured in BRIDGES' 1938 map as a doublet, it most frequently, if not invariably, appears as a single fairly heavy band, adhering closely to the 1 B 3·4 doublet. The width and

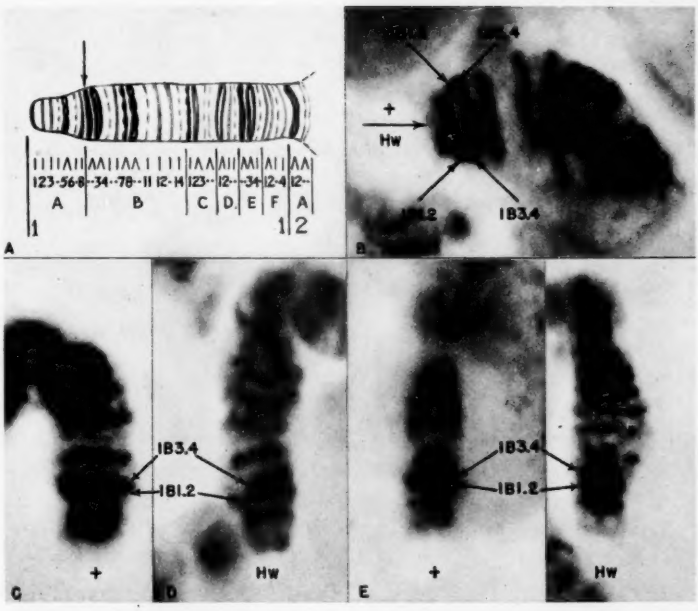

FIGURE 1.—(a) Map of the tip section of the X chromosome (BRIDGES 1938). (b) Synapsed tip of X heterozygous for *Hw* (*ct* 268–13 *y Hw*/*ct v dy gf*) showing the 1 B 1·2 band much heavier in the *Hw* strand than in the + strand. (c) and (d) + and *Hw* strands from the same nucleus of *N*264–30/d1–49, *y Hw* material, showing in the *Hw* strand the 1 B 1·2 band separated from the 1 B 2·3 band and heavier than in the + strand. (e) and (f) Same as (c) and (d) but for *w* 258–18/d1–49, *y Hw* material.

relative intensity of this band as seen in a well stretched normal chromosome is presented in figure 1a. As a comparison, figure 1b shows a diploid chromosome in which it will be noted that the 1 B 1·2 band of one strand is much heavier than that of the other. The appearance shown here is fairly typical for material heterozygous for *Hw*. The comparison is even more striking when the strands are separate, for then not only is the difference in the intensity of the 1 B 1·2 band noticeable but the change in the average spacing between 1 B 1·2 and 3·4 is also evident. In the *Hw* haploid the two doublets appear to be of equal thickness and are quite widely separated. This comparison can be made by referring to figure 1c which shows a normal and figure 1d which shows a *Hw* strand from the same nucleus of the *N* 264-30/dl-49, *y Hw* material described above, and to figures 1e and 1f similarly showing the two haploids from the same nucleus of 258-18/dl-49, *y Hw* material.

From this study it may be concluded that *Hw* is cytologically visible as a duplication for the band 1 B 1·2 and that this duplication is most clearly evident in haploid strands which show both the difference in the width of the band and its space relationship to 1 B 3·4 as compared with its appearance in normal strands.

GENETIC RESULTS

The translocation *w* 258-18 (DEMEREC and SLIZYNSKA 1937) is a reciprocal translocation between the X and fourth chromosomes. The break in the X is located between *rst* and *N* and in the fourth in the chromocentral region. Thus the tip section of the X chromosome is attached to the spindle fiber of the fourth. Since individuals lacking the fourth chromosome are able to live, this tip can be established as an independent chromosome and when present with the normal X it produces a duplication for the section up to and including *rst*. It has been noticed that males carrying this duplication, namely, those which have in addition to the whole X chromosome an extra piece from the tip to *rst*, exhibit certain characteristics of *Hw*. Such males invariably have extra occipital bristles and in addition they frequently show extra bristles on the thorax and along the veins on the wings. Females with two full X chromosomes and a duplicated piece also show certain characteristics of *Hw*. As a rule one or both occipital bristles are present; sometimes extra bristles appear on the thorax, but extra hairs on the wings have not been observed in the material examined.

A similar hairy wing effect is visible in two other duplications for the tip of the X chromosome which have been found at our laboratory. In both of these the break in the X chromosome occurred in approximately the same region as in *w* 258-18 and thus in all three cases a similar piece of the

chromosome is duplicated. All this evidence indicates that the dominant hairy wing effect may be due to a duplication for a certain region of the tip of the X chromosome and also that the *Hw* gene may be nothing else but such a duplication carried within one chromosome. Cytological observations reported above show that a chromosome carrying the *Hw* mutant carries a cytological duplication for the 1 B 1·2 band which suggests that this duplication is responsible for the hairy wing effect.

While this work was in progress, our attention was called to the fact that other known duplications for the tip region also exhibit the hairy wing effect, as is to be expected if a duplication of a certain sector is responsible for the appearance of that character. In our experiments we used the short duplication 118 found by DOBZHANSKY (1932) rather than our longer duplications in order to reduce the possible effect of additional regions of the chromosome. In that duplication the break is located between *svr* and *br*.

Males having in addition to the normal X and Y chromosomes either our duplication *w* 258-18 or DOBZHANSKY's duplication 118 frequently have extra hairs on the wings. These are usually located on the distal section of the second vein and in the cells surrounding it. Such males regularly have either one or two occipital bristles while wild-type flies have none. They also have an increased number of hairs on the back of the head. Females with two normal X chromosomes and such a duplication regularly have either one or two occipital bristles and an increased number of hairs on the back of the head, while extra hairs on the wings have not been observed on such females.

Working on the assumption that the 1 B 1·2 band, when doubled, is responsible for the hairy wing effect, an attempt was made to obtain quantitative data regarding the effect of various doses of the 1 B 1·2 band. The number of extra hairs present on the wings was used as a criterion. One hundred flies were taken from each lot, and the hairs on the upper surface of the right wing counted. Means of these counts are given in table 1. The same table gives observations on the occipital bristles and the second column shows the number of 1 B 1·2 bands in each of the X chromosome tips present in the combination. The various combinations recorded here were derived from experiments in which *y w*, Df 260-2/*y Hw w*, and *y w*/Dp 118 stocks were used.

It is known that *Hw* is a very variable character, due to various genetic and environmental causes. In these experiments no attempt was made to control the genetic cause of variability, but in order to reduce environmental differences all cultures used in counts were raised at 22°C.

Salivary gland chromosome studies indicate that *ac* is located in the region following the band 1 A 4 and preceding the band 1 B 3·4 (DEMEREC

TABLE 1

Average number of setae on flies carrying various combinations of 1 B 1·2 bands. (Hw=Hairy wing; Df=deficiency 260-2; Dp=duplication 118; +=normal chromosome.)

GENETIC CONSTITUTION	NUMBER OF 1 B 1·2 BANDS	EXTRA HAIRS ON WINGS	NUMBER OF OCCIPITALS
Females			
+/+	1/1	0	0
Df/Df/Dp	0/0/1	0	0
Df/+/Dp	0/1/1	0	0
+/+/Dp	1/1/1	0	1–2
Hw/Df	2/0	17.65±0.38	2
Hw/+	2/1	17.05±0.56	2
Hw/Df/Dp	2/0/1	16.05±0.24	2
Hw/+/Dp	2/1/1	18.06±0.26	2
Hw/*Hw*	2/2	21.17±0.43	2
Hw/*Hw*/Dp	2/2/1	32.96±0.73	2
Males			
+	1	0	0
Df/Dp	0/1	0	0
+/Dp	1/1	0+	1–2
Hw	2	13.56±0.27	2
Hw/Dp	2/1	17.37±0.30	2

and HOOVER 1936). In this section of the chromosome, the band, 1 B 1·2, which is duplicated in *Hw*, is present. Since both *ac* and *Hw* are located in the same section of the chromosome and since both of them affect setae, it became evident that these two genes might possibly be alleles; namely, that *Hw* might be a duplication for *ac*. Thus, the experiments reported in table 2 were conducted to determine the behavior of these two characters in various combinations. *Hw* increases the number of thoracic bristles and in particular the number of dorsocentrals. Achaete, on the other hand, reduces the number of dorsocentral bristles, posterior dorsocentrals being

TABLE 2

Average number of dorsocentral bristles on females differing in genetic constitution in regard to Hairy wing and achaete.

GENETIC CONSTITUTION	AVERAGE NUMBER OF DORSOCENTRALS	
	ANTERIOR	POSTERIOR
Hw +	4.90	3.89
Hw/*ac*	3.87	3.72
Hw/Df	3.68	3.50
+/+	2.00	2.00
ac/*ac*	2.00	0.39
ac/Df	2.00	0.21

particularly affected. The average number of dorsocentrals, as determined on one hundred females, was used as a criterion in the measurements reported in table 2. It is evident from these data that *Hw* is affected by *ac*, as might be expected if they were alleles.

DISCUSSION

Cytological evidence indicates that in the *Hw* chromosome the 1 B 1·2 band in the tip region is represented twice. Since genetic evidence shows that flies carrying any of the several available duplications for that region exhibit certain characteristics of *Hw*, the conclusion seems justifiable that the duplication for the 1 B 1·2 band is responsible for the appearance of the dominant *Hw* character. That this band may represent the *ac* locus is suggested by the following lines of evidence: (1) The cytogenetic study of three terminal deficiencies (DEMEREC and HOOVER 1936) shows that *ac* is located in this particular region of the chromosome, that is, in the section represented by seven bands, 1 A 4 to 8 and 1 B 1·2. (2) Both *Hw* and *ac* affect bristles; *Hw* increases their number while *ac* suppresses them. (3) Data given in table 2 indicate that *ac* affects *Hw* and also that it changes it in the same direction as a deficiency for that region, indicating that *ac* and *Hw* may be alleles.

Until now, Bar (*B*) was the only known case of a dominant mutation produced by a tandem duplication of a minute segment. As shown by BRIDGES (1936), *B* is a duplication for six bands. It is well known from studies made by STURTEVANT (1925) that crossing over may occur between two homologous chromosomes each carrying *B*, and thus one chromosome with two *B*'s is produced. STURTEVANT showed that two *B*'s located in the same chromosome have a more pronounced effect than two *B*'s located in different chromosomes, and he attributed this to position effect. When it was found that *Hw* is a tandem duplication, it became of interest to know if the relationships observed in the *B* case exist also in *Hw*. Unfortunately crossing over has not been observed in the *Hw* region of the chromosome, so that a series parallel to *B* cannot be obtained for *Hw*. As already mentioned, single *B* has a set of six bands represented twice and consequently double *B* should have them represented three times in the same chromosome. However, short duplications which are available for the *Hw* region make possible combinations which, although they do not duplicate the *B* set-up, parallel it sufficiently to make a comparison feasible. The data given in table 1 show that *Hw* behaves similarly to *B*: the duplication when present in one chromosome exhibits a stronger effect than if present in two separate chromosomes. For example, females with three sets of the 1 B 1·2 band divided among three chromosomes had no extra hairs on wings, while flies with three sets divided between two chromosomes had 17.05 extra hairs. Similarly, flies with four sets of bands

distributed among three chromosomes had 18.06 hairs, and distributed between two chromosomes had 21.17 hairs. A similar situation was observed in males.

It should be pointed out that the duplications used in our experiments contain in addition to the band 1 B 1·2 an appreciable number of adjacent bands, and the possibility that the effect described above might be due to that extra material is not excluded. Although this possibility exists, it seems that the interpretation discussed above is more plausible.

It has already been pointed out that while crossing over between two *B* duplications occurs readily, no case of crosing over was observed between the bands forming the *Hw* duplication. The closeness of these two bands may not be the only factor which is responsible for this behavior. It is known that crossing over is not always a function of distance. Regions are known, such as the white-facet region of the X chromosome, where crossing over is relatively frequent and also regions are known where crossing over is rare. The yellow-scute segment, where *Hw* is located, is such a region with a low frequency of crossing over.

SUMMARY

A study of salivary gland chromosomes indicates that in the *Hw* chromosome the band 1 B 1·2 is represented twice. Genetic evidence shows that flies carrying duplications for the tip of the X chromosome, where *Hw* is located, exhibit certain characteristics of Hairy wing. Thus, the conclusion is drawn that the dominant *Hw* character is a duplication for the 1 B 1·2 band.

Experiments involving *ac* suggest that this locus is involved in the *Hw* duplication.

Genetic tests indicate that the phenotypic effect is stronger if two 1 B 1·2 bands are located in one chromosome than if they are distributed between two chromosomes.

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DOMINANCE MODIFIERS OF SCUTE IN DROSOPHILA PSEUDOOBSCURA

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Received November 28, 1938

INTRODUCTION

THE question of the nature and origin of dominance has been much discussed in recent years. Two of the best motivated theories have been advanced by FISHER (1928a, 1928b, 1929, 1931, 1934) and by WRIGHT (1929a, 1929b, 1934a, 1934b) and HALDANE (1930, 1932, 1933) respectively. Views essentially similar to WRIGHT'S and HALDANE'S were expressed also by MULLER (1932) and PLUNKETT (1932). FISHER'S contention is that dominance has evolved as a result of the action of many small modifying genes. He believes that mutations arising for the first time in the history of the species are, as a rule, neither dominant nor recessive. It is the action of specific groups of modifying genes, built up as a result of a long selection process, which determines the expression of a mutant gene in heterozygous condition. WRIGHT and HALDANE suppose that evolution of the dominance of a gene is a function of the physiological action of this gene itself. They contend that genes act to produce a character by means of enzymes; if any link in the chain of reactions producing some character is defective in any way, the net result will be disastrous to the end-product. Therefore, those genes which produce an excess of enzyme which would be great enough to withstand adverse changes in the environment, would be selected because of their own survival value. In such a way the wild genotype would gradually become dominant to the frequently recurring mutations.

It was hoped that some experimental evidence bearing on the above problems might be obtained in *Drosophila pseudoobscura*. It is known that *D. pseudoobscura* exists in two races which have been designated as A and B; furthermore many wild strains which have been obtained from different parts of the geographical area of the species are available. From some preliminary investigations of PROFESSOR A. H. STURTEVANT (unpublished), it was known that various strains of both race A and race B differ in their reactions to the race A semidominant mutation Scute. Accordingly, at the suggestion of PROFESSOR STURTEVANT, it was decided to investigate in more detail the manifestation of the gene Scute in heterozygotes with various different strains of race A as well as race B.

MATERIAL AND METHOD

Various wild strains of race A and of race B of *Drosophila pseudoobscura* were obtained through the courtesy of PROFESSOR TH. DOBZHANSKY.

Strains from widely separated areas were taken, so as to increase the probability of obtaining widely different sets of modifying genes, and to see if any relation existed between the geographical origin of a strain and its reaction to the degree of dominance of Scute. These wild strains were crossed to the standard mutant stock, *orange Scute purple*. These mutants, *or*, *Sc*, and *pr*, all lie in the third chromosome. This standard testing stock belongs to race A. As Scute is more or less dominant, the first generation offspring were counted in all cases. Reciprocal matings were made for the first few series of crosses. Later this was deemed no longer necessary and it was discontinued. It may be mentioned that the gene Scute of *D. pseudoobscura* is not homologous to that of *D. melanogaster*. The *D. pseudoobscura* mutation affects all the bristles on the head, thorax, and scutellum in a rather haphazard fashion, cases of asymmetry being frequent. Also, it is located in the third chromosome, whereas the scute of *D. melanogaster* is a sex-linked recessive affecting various bristles in a definite pattern. For the purposes of the present investigation, however, only the four dorsocentral and four scutellar bristles were counted. The usual procedure was to note the number present, making no attempt to distinguish between them.

The temperature was maintained as constant as possible by keeping the cultures in an incubator designed by BRIDGES (1932). The temperature was maintained at 24°C. In order that all external variations be minimized as completely as possible, the flies from which the eggs were to be obtained were transferred to fresh bottles every 24 hours. Only flies from bottles containing between 25 and 150 individuals were used for counts. Most of the bottles produced about 50 to 100 flies. This rule was rigidly adhered to in order to remove any influences that under- and over-population might have on bristle formation. Flies were examined within one or two days after hatching to lessen the chance of any error induced by possible oversight of broken-off bristles; older flies often lose many bristles in this way, and, due to the extremely dark thorax of *D. pseudoobscura*, a certain amount of misclassification may occur.

DOMINANCE OF SCUTE IN HYBRIDS WITH VARIOUS STRAINS

A summary of the results of bristle counts in the hybrids between *or Sc pr* and various wild strains is presented in tables 1 and 2. The name of each strain indicates the geographical locality in which its wild ancestor was collected. (For further information regarding the origin of these strains the papers by DOBZHANSKY 1935, 1937, and DOBZHANSKY and STURTEVANT 1938, may be consulted.) The tables show the mean bristle numbers with their standard errors ($M \pm m$), and the numbers of individuals studied (n) to obtain these means. It is quite evident that the

degree of the dominance of Scute is not alike in different hybrids. The hybrids between *or Sc pr* and Merritt-2, for example, have 3.01 and 3.23 bristles present in females and males respectively, while the *or Sc pr*×Henshaw-3 hybrids have only 1.21 bristles in females and 1.35 bristles in the males (table 2, first experiment). Thus, Scute is more nearly dominant in hybrids with Henshaw-3 than in those with Merritt-2. Other strains give results intermediate to those observed when Henshaw and Merritt are used, the variation apparently being continuous. The numbers of bristles present in female and in male hybrids from a given cross are, on the whole, constant. The males, however, tend to have somewhat more bristles than do females. The minor discrepancies observed are mostly within the limits of the sampling errors.

Since the bristle number is a character that seems to be highly sensitive to culture conditions, repeated tests were made using in part the same strains (tables 1 and 2). The results show that the differences observed are undoubtedly real and not accidental; thus, in every instance the hybrids with Merritt have more bristles than those with Henshaw. An attempt to find a correlation between the degree of the dominance of Scute in the hybrids with a given strain and the geographical origin of the latter proved unsuccessful. For example, some of the strains from British Columbia, which is the extreme northern part of the distribution of *D. pseudoobscura*, gave some weak (Merritt-2, Nakusp-3), and others strong (Kamloops, Mara-3) dominance of Scute. Race A and B show no consistent differences in this respect (compare Grand Canyon-3 and Henshaw-3, Lassen-8 and Crater Lake-2). We can not, of course, exclude the possibility that the populations of either race inhabiting some small areas may give a consistently high or consistently low degree of dominance in hybrids with Scute, but there is certainly no pronounced geographical trend for the species or a race as a whole in this respect. Such geographical trends have been observed, however, for some other variable genetic characters in *D. pseudoobscura* (sex-ratio, STURTEVANT and DOBZHANSKY 1936; the gene arrangement in the third chromosome, DOBZHANSKY and STURTEVANT 1938; the "strength," DOBZHANSKY, unpublished, and others).

Reciprocal crosses (*or Sc pr* ♀ ×wild ♂ and wild ♀ ×*or Sc pr* ♂) were arranged for some strains. The comparison of the results of such reciprocal crosses might be of interest as a method for the detection of sex-linked genes modifying the dominance of Scute. Indeed, the male hybrids from the *or Sc pr* ♀ ×wild ♂ crosses have always the X chromosome of the *or Sc pr* strain and the Y chromosomes of the wild ones; those from the reciprocal crosses have different X chromosomes depending upon the wild strain used, and the Y chromosome of the *or Sc pr* parent. Comparison of table 1 with table 2 shows that in the wild ♀ ×*or Sc pr* ♂ cross, the

TABLE 1

Mean number of bristles present in F_1 offspring from the cross of or Sc pr ♀ × wild strain ♂.

STRAIN	RACE	FEMALES		MALES	
		M ± m	n	M ± m	n
Experiment 1 11/29/36–12/2/36					
Merritt-2	A	3.01±0.08	306	3.23±0.10	334
Nakusp-3	A	2.51±0.10	212	2.04±0.10	180
Chelan-10	A	1.72±0.12	96	1.38±0.11	111
Campbell-3	B	1.57±0.11	115	1.58±0.14	80
Chelan-2	A	1.54±0.10	205	1.63±0.08	251
Chelan-7	A	1.34±0.10	133	1.16±0.10	126
150-Mile House-5	B	1.29±0.11	76	1.71±0.12	99
Sisters-9	B	1.26±0.14	53	1.31±0.14	61
La Push-4	B	1.23±0.09	167	1.41±0.11	134
Henshaw-3	A	1.21±0.06	266	1.35±0.08	228
Experiment 2 1/20/37–1/28/37					
Grand Canyon-3	A	2.60±0.12	157	2.31±0.14	132
Merritt-2	A	2.29±0.10	221	2.62±0.11	156
Lassen-8	B	2.07±0.10	123	1.72±0.11	118
La Grande-2	A	1.94±0.09	193	1.97±0.10	158
Cuernavaca-2	A	1.82±0.12	193	2.70±0.18	100
Oaxaca-4	A	1.76±0.09	199	1.72±0.09	187
Taos-1	A	1.65±0.09	203	1.71±0.11	139
Campbell-3	B	1.63±0.11	102	1.34±0.11	122
Big Horn-6	A	1.55±0.07	224	1.39±0.09	171
Sequoia-15	A	1.54±0.09	171	1.36±0.10	112
Oaxaca-5	A	1.53±0.09	154	1.76±0.10	131
Estes Park-1	A	1.18±0.10	143	1.31±0.10	113
Sequoia-8	B	1.14±0.10	140	1.37±0.11	134
Julian W-5	A	1.13±0.08	183	1.08±0.08	186
Santa Lucia-11	B	1.02±0.08	143	1.18±0.09	134
Crater Lake-2	B	0.97±0.12	66	1.31±0.13	63
Henshaw-3	A	0.96±0.08	139	1.46±0.10	125
Experiment 3 3/16/37–3/25/37					
Chiricahua-2	A	4.31±0.11	165	4.72±0.15	114
Grand Canyon-3	A	3.01±0.21	93	2.18±0.19	105
Julian E-6	A	2.57±0.12	166	2.64±0.11	165
Merritt-2	A	2.30±0.11	169	2.45±0.14	137
Arrowhead	A	2.29±0.19	116	2.45±0.18	126
Dollar Lake-2	A	2.09±0.08	236	2.07±0.10	139
Barton Flats-9	A	2.06±0.10	161	1.67±0.10	152
Magdalena-2	A	1.74±0.10	151	1.53±0.10	133
Kamloops	A	1.72±0.11	111	1.74±0.11	110
Black Hills-5	A	1.71±0.10	142	1.85±0.09	150
Big Bear-2	A	1.70±0.10	148	1.73±0.11	115
Mara-3	A	1.48±0.12	116	1.05±0.10	116
Durango-3	A	1.46±0.08	227	1.39±0.11	97
San Gabriel-7	A	1.44±0.11	140	1.52±0.10	139
Skaha-8	A	1.43±0.08	177	1.73±0.11	133
Henshaw-3	A	1.43±0.09	171	1.51±0.10	136
Zion-5	A	1.34±0.09	157	1.34±0.09	149
Guadelupe-5	A	1.31±0.08	164	1.19±0.08	147
Skaha-2	A	1.26±0.09	149	1.31±0.10	141
Pavilion	A	0.99±0.09	126	0.83±0.08	108

male hybrids have consistently higher mean bristle numbers than the female hybrids; in the reciprocal crosses (table 1) the numbers of bristles

TABLE 2

Mean number of bristles present in F_1 offspring from wild-type females× or Sc pr males.

STRAIN	RACE	FEMALES		MALES	
		M±m	n	M±m	n
Experiment 1 12/19/36–12/24/36					
Chelan-10	A	2.69±0.11	151	5.96±0.16	75
Merritt-2	A	2.55±0.09	231	3.44±0.14	176
Nakusp-3	A	2.52±0.12	151	4.25±0.14	126
Oaxaca-4	A	2.28±0.14	130	3.28±0.19	87
Campbell-4	B	2.05±0.12	157	2.83±0.12	115
Chelan-2	A	2.00±0.08	212	2.86±0.10	193
Texas	A	1.96±0.16	98	3.31±0.16	67
Sequoia-8	B	1.78±0.11	114	2.96±0.16	73
Chelan-7	A	1.60±0.12	132	3.60±0.15	102
150-Mile House-5	B	1.52±0.08	199	2.90±0.13	107
La Push-4	B	1.52±0.09	165	3.78±0.13	156
Henshaw-3	A	1.22±0.09	103	2.62±0.20	63
Experiment 2 3/7/37–3/10/37					
Grand Canyon-3	A	3.26±0.15	132	5.19±0.15	113
Merritt-2	A	2.95±0.13	139	3.94±0.14	116
La Grande-2	A	2.35±0.10	168	3.25±0.12	143
Oaxaca-5	A	1.58±0.10	165	3.15±0.13	137
Julian W-5	A	1.47±0.13	84	2.62±0.24	69
Estes Park-1	A	1.43±0.10	156	2.23±0.16	126
Henshaw-3	A	1.26±0.09	136	2.13±0.11	126
Taos-1	A	1.25±0.10	120	2.04±0.12	121
Sequoia-15	A	1.25±0.10	102	2.51±0.13	115
Cuernavaca-2	A	1.19±0.09	153	2.42±0.13	164
Big Horn-6	A	1.11±0.12	79	2.20±0.14	81

in females are, as a rule, only slightly lower than in the males. It appears either that the Y chromosome of the *or Sc pr* strain has modifiers lessening the dominance of Scute, or that the X chromosome of the same strain has genes enhancing its dominance. The effects of the presumed modifiers located in the X or Y chromosomes of various wild strains might express themselves in shifting the positions of males in the tables with respect to that of the females. It can be seen that in tables 1 and 2 the strains are arranged according to the diminishing bristle numbers in the females; on the whole the seriation of the number of bristles in the males coincides with that for the females, indicating that the dominance modifiers are either absent in the X and Y chromosomes of the wild strains, or that their relative strengths in these chromosomes and in the autosomes are alike. In a few instances this is not so, however. For example, in the *or Sc pr*

♀ ×wild ♂ crosses (table 1), the hybrids with the 150-Mile House strain show an equal or smaller number of bristles in the females than the hybrids in which the Campbell-3, Chelan-2, and Chelan-7 strains took part Yet, the cross *or Sc pr* ♀ ×150-Mile House-5 ♂ produces males with more bristles than similar crosses to Campbell and Chelan. Similar "shifts" are observed for Cuernavaca-2 (second experiment) and for Mara-3 and Skaha-8 (third experiment, table 1). This suggests that modifiers in the Y chromosome may be the cause. Among the wild-type ♀ ×*or Sc pr* ♂ crosses, "shifts" are observed for Nakusp-3 and La Push-4 hybrids (table 2), indicating dominance modifiers in the X chromosome.

The validity of the above conclusions is nevertheless questionable due to the high sensitivity of the bristle number to environmental influences. As a control we may use comparisons of the bristle numbers in the female hybrids in reciprocal crosses. The genetic constitution of females obtained in reciprocal crosses is evidently alike. Nevertheless, in the *or Sc pr* ♀ ×wild ♂ crosses, the strains Cuernavaca-2 and Big Horn-6 gave rather high, and in the reciprocal crosses low, bristle numbers (compare tables 1 and 2). Repeated tests were made for these strains, the reciprocal crosses being arranged simultaneously. As shown in table 3, this time no difference between the crosses was observed, although the dominance of Scute in Cuernavaca-2 hybrids was lower than in those with Big Horn-6.

TABLE 3

Repeated reciprocal comparisons giving mean number of bristles present in F_1 females.

STRAIN	RACE	*or Sc pr* ♀ ×+♂		+♀ ×*or Sc pr* ♂	
		M±n	n	M±n	n
Grand Canyon-3	A			2.84±0.15	149
Merritt-2	A			2.66±0.11	196
Cuernavaca-2	A	2.10±0.14	117	2.31±0.11	156
Big Horn-6	A	1.34±0.10	110	1.59±0.09	162
Henshaw-3	A	1.15±0.09	120	1.85±0.10	147

A perusal of the data (tables 1, 2 and 3) also shows that F_1 females from the cross *or Sc pr* ♀ ×wild strain ♂ (table 1), tend to have fewer bristles than do the F_1 females from the reciprocal cross (table 2). As these females differ only in the source of their cytoplasm this may be explained as due to a maternal effect. Since the data are not comprehensive enough this conclusion can not be regarded as established at present.

IS THERE A VARIETY OF WILD-TYPE ALLELES OF SCUTE?

The experiments discussed above show conclusively that the degree of the dominance of Scute in hybrids with different wild strains is variable.

One may now inquire what causes are responsible for these variations. Theoretically, three possibilities present themselves. First, the wild-type alleles of Scute found in different wild strains may not all be alike, some possibly being more and others less recessive with respect to the mutant gene Scute. Second, the wild strains tested may carry at loci other than Scute different modifying genes which influence the manifestation of the latter in heterozygotes. These modifiers may be present in the third as well as in other chromosomes. The third possibility is that the phenomena observed are due to the presence of a variety of wild-type alleles at the Scute locus as well as to the dominance modifiers at other loci. The experiments to be described below were designed to discriminate between these possibilities.

To reveal the presence of different wild-type alleles of Scute, strains must be obtained that differ only in the origin of their Scute locus, and that are otherwise isogenic. Strictly speaking, this requirement is unattainable since there is no technique available that would permit the creation of completely isogenic strains. One may, however, try to approach this condition as closely as possible. The Merritt-2 and Henshaw-3 strains are known to give different numbers of bristles if crossed to the *or Sc pr* strain. Merritt-2 and Henshaw-3 were outcrossed, therefore, to a strain homozygous for the third chromosome recessive genes orange (*or*), polychaete (*po*), purple (*pr*), and crossveinless (*cv*). Polychaete is a recessive allele of Scute. The *or*, *pr* and *cv* genes lie in the same chromosome to the left and to the right of the *Sc* locus respectively. The hybrid females were back-crossed to *or po pr cv* males, and crossover chromosomes were obtained which had the middle part (carrying the wild-type allele of *Sc* and *po*) from the Merritt or Henshaw strain, and the remainder from *or po pr cv* strain. Four more back-crosses to the *or po pr cv* strain were carried out to make it probable that in the resulting strains all the Merritt and Henshaw chromosomes were replaced by the homologues from the *or po pr cv* strain. Finally, the *or*+^{sc} (Henshaw) *pr cv*/*or po pr cv* and *or*+^{sc} (Merritt) *pr cv*/*or po pr cv* males were crossed to *or Sc pr* females. A scheme of these crosses is represented in figure 1.

Two classes of flies appear in the offspring: those heterozygous for Scute and the +^{sc} alleles of Merritt and Henshaw respectively, and those carrying *Sc* and *po*. They can be distinguished because of the fact that *Sc*/*po* flies have rough eyes. The results of the bristle counts are summarized in table 4. One may see that the numbers of bristles present in +^{sc} (Merritt)/*Sc* and +^{sc} (Henshaw)/*Sc* flies of either sex are alike. This shows that there is no appreciable difference between the wild-type alleles of *Sc* present in the Merritt-2 and Henshaw-3 strains.

Another experiment, similar in principle to the one just described, was

carried out to compare the wild-type alleles of Scute present in race A and race B. A strain obtained from PROFESSOR TH. DOBZHANSKY had one race-A third chromosome carrying *Sc* and *pr* on an otherwise pure race-B background (figure 2). This strain was maintained by crossing *Sc pr*

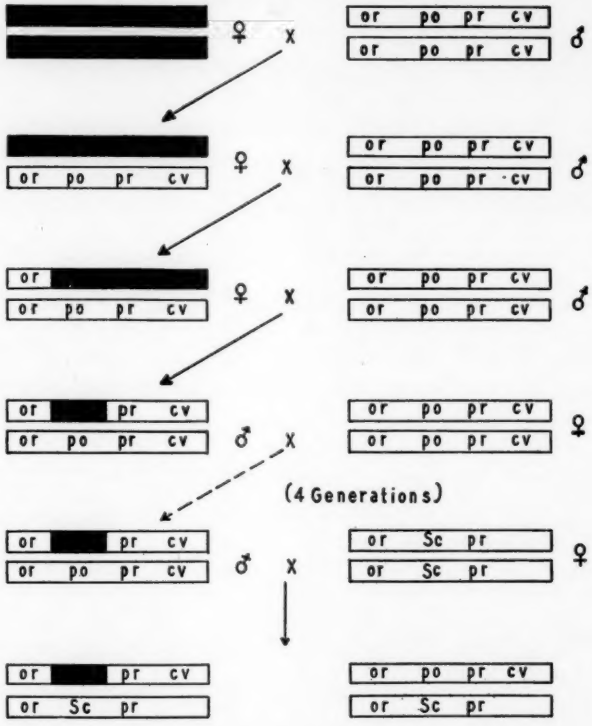

FIGURE 1.—Diagram of the third chromosomes of *D. pseudoobscura* showing method of testing for the possible differences between the Scute loci of Merritt and Henshaw strains. Black = Merritt or Henshaw chromosomes. White = neutral strain.

(race A)/*or* (race B) females to homozygous *or* (race B) males in every generation. Since the third chromosomes of race A and B differ in an inverted section (TAN 1935), the frequency of crossing over between the two chromosomes is very low, and the presence of the marking genes permits keeping the third chromosome of race A intact. The number of bristles present in flies of this strain was very high, namely 5.77 ± 0.08 (table 5,a). A crossover was obtained which appeared wild-type in phenotype (figure 2), but which must have had all race-B chromosomes except

TABLE 4

Mean number of bristles present in or Sc pr ♀ × or +Henshaw pr cv/or po pr cv ♂ and or Sc pr ♀ × or +Merritt pr cv/or po pr cv ♂ crosses.

TYPE OF OFFSPRING	FEMALES		MALES	
	M ± m	n	M ± m	n
(a) +Merritt/*Sc*	1.16±0.09	159	1.39±0.12	93
(b) +Henshaw/*Sc*	1.21±0.10	137	1.43±0.12	86
(a′) *po*/*Sc*	2.83±0.09	148	2.06±0.11	95
(b′) *po*/*Sc*	3.03±0.09	160	2.22±0.15	87

for a short section of the third chromosome containing the wild-type allele of orange coming from race A. This crossover fly (a male) was outcrossed to pure race A females homozygous for orange and purple. A non-orange female was selected in the next generation and again crossed to *or pr* race-A males. This was repeated for two more generations. As a

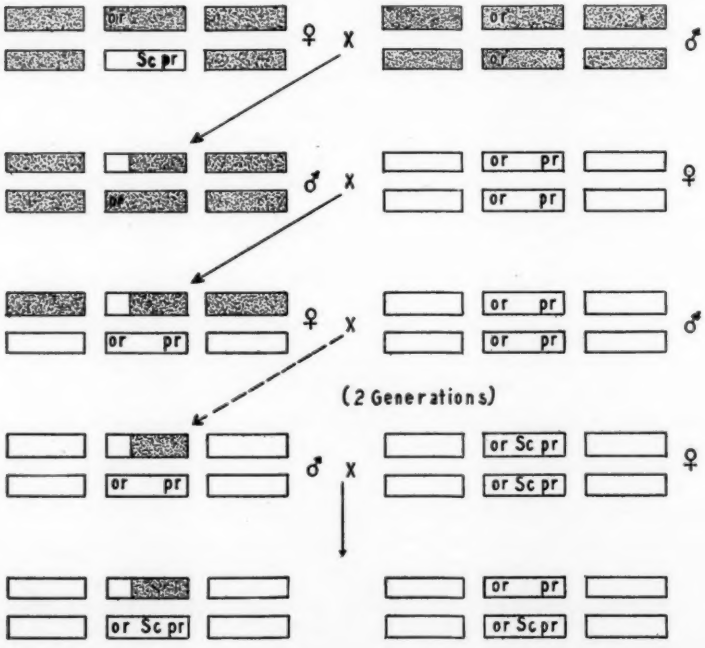

FIGURE 2.—Diagram showing method used to determine the possible differences between the Scute locus of race A and race B of *D. pseudoobscura*. Stippled = race-B autosomes. White = race-A autosomes.

result of this procedure, flies were obtained that had race-A chromosomes except for a section of the third containing the wild-type alleles of Scute and purple (figure 2). Males of this constitution were then crossed to *or Sc pr* race-A females. Two types of flies appear in the offspring, namely *or Sc pr*, and *Sc*. Both types are heterozygous for *Sc*, but the first of them has only race-A chromosomes while the second carries a wild-type allele of Scute from race B. The number of bristles present in these flies is shown in table 5 under (b) and (c) respectively. These numbers are not significantly different, showing that the wild-type alleles of Scute present in the particular race-A and race-B strains studied are virtually alike.

Two other sets of figures are also included in table 5. Under (a) is shown the number of bristles present in the heterozygous Scute flies from the original strain of DOBZHANSKY (see above, also the upper line of figure 2). Under (d) is shown the number of bristles present in the F_1 hybrids from the cross of *or Sc pr* race A ♀ ×orange race B ♂. The *or* race-B strain used here is the same one as that applied by DOBZHANSKY in his crosses. The number of bristles in (a) and (d) are very different indeed, suggesting that the orange race-B strain carries recessive modifiers tending to suppress the effect of Scute. Obviously, these modifiers must be located in chromosomes other than the third.

TABLE 5

Mean number of bristles present in flies carrying race A and race B wild-type alleles of Scute respectively.

STRAIN	FEMALES		MALES	
	M±n	n	M±n	n
(a) *Sc pr/or*	5.77±0.08	273	5.73±0.08	267
(b) *or Sc pr/+B⁺+*	2.45±0.13	383	3.26±0.17	244
(c) *or Sc pr/or A⁺ pr*	2.59±0.13	348	3.01±0.16	186
(d) *Sc/or B*	1.41±0.13	139	1.26±0.17	93

DOMINANCE MODIFIERS IN THE X CHROMOSOME

An experiment was arranged to test the possible differences in the X chromosomes of two widely divergent strains, Merritt and Henshaw. The strain Merritt-2 is known to give high numbers of bristles in hybrids with *or Sc pr*, while the Henshaw-3 strain gives low bristle counts in the same hybrids. Reciprocal crosses of Merritt ♀ ×Henshaw ♂ and Henshaw ♀ ×Merritt ♂ were made. The males obtained in the offspring of the first of these crosses carry an X chromosome of the Merritt strain and a Henshaw Y. The males from the second cross have a Henshaw X and a Merritt Y chromosome. As far as the autosomes are concerned, the two types of males are alike. Now, these males were outcrossed to *or Sc pr*

females, and bristle counts were made in the offspring (table 6). If the genes modifying the expression of Scute located in the X chromosome were dominant to those in the *or Sc pr* X chromosome, the daughters of the (Merritt ♀ × Henshaw ♂) males must have more bristles than the daughters of the (Henshaw ♀ × Merritt ♂) fathers. Table 6 shows that this is certainly not the case. A similar experiment was arranged using the strains Big Horn-6 (low) and Grand Canyon-3 (high bristle numbers). The results obtained (table 6) may be interpreted as indicating the presence of some modifiers in the X chromosome dominant to those in the X chromosome of the *or Sc pr* strain, but in any event the difference observed here is much smaller than that found in *or Sc pr* × Grand Canyon and *or Sc pr* × Big Horn crosses (tables 1 and 2).

TABLE 6

Mean number of bristles present in the offspring of the following four crosses.

CROSS	FEMALES		MALES	
	M±m	n	M±m	n
or Sc pr ♀ × (Merritt-2 ♀ / Henshaw-3 ♂) ♂	4.73±0.11	525	5.45±0.19	145
or Sc pr ♀ × (Henshaw-3 ♀ / Merritt-2 ♂) ♂	5.03±0.11	417	5.15±0.20	118
or Sc pr ♀ × (Grand Canyon-3 ♀ / Big Horn-6 ♂) ♂	4.05±0.13	387	3.43±0.22	138
or Sc pr ♀ × (Big Horn-6 ♀ / Grand Canyon-3 ♂) ♂	3.54±0.11	487	3.40±0.21	123

DOMINANCE MODIFIERS IN THE AUTOSOMES

By the process of elimination of other possibilities we are forced to conclude that the variation of the bristle numbers observed in hybrids between *or Sc pr* and different wild strains is due to the presence of dominance modifiers in the autosomes of these strains. The experimental procedure employed to test the validity of this conclusion is as follows (figure 3). The mutant genes Smoky (*Sm*), orange (*or*), and Curly (*Cy*) were used as markers for the second, third, and fourth chromosomes respectively. Merritt-2 and Henshaw-3 females were crossed to *Sm or Cy* males. Sons showing *Sm* and *Cy* (and heterozygous for orange) were selected in the offspring and outcrossed to *or Sc pr* females. In the next generation, eight classes of flies appear showing all the combinations of the marking genes *Sm*, *or*, and *Cy*. The presence or absence of these markers show which, if any, of the autosomes coming from the Merritt and Henshaw strains are carried by the fly in question. Thus, a *Sc/Sm or Cy* fly carries no chromosomes from the strain to be tested; a *Sc/or Cy* fly carries only the second chromosome; a *Sc/Sm* fly has the third and the fourth chromosomes of Merritt or Henshaw, etc. (see figure 3). A summary of mean bristle counts in all types of flies obtained in these crosses is given in table 8, for females and males separately.

The experiment is thus reduced to comparisons of the effects of individual chromosomes of the Merritt and Henshaw strains with those of their homologues in the *or Sc pr* strain. For example, if the second chromosome of the Merritt strain carries modifiers increasing the number of

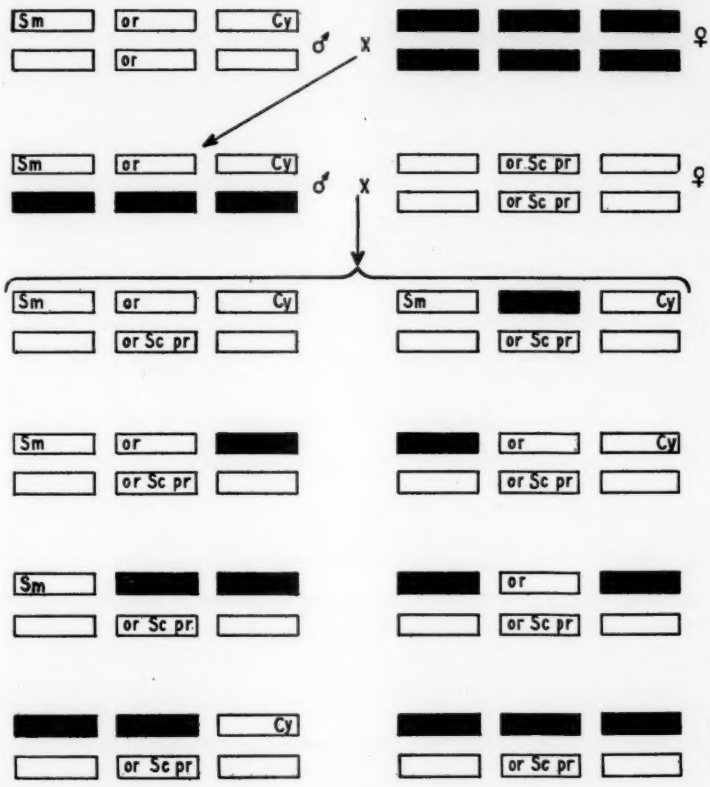

FIGURE 3.—Diagram showing method used to test the individual autosomes of wild strains for dominance modifiers of Scute. Black = autosomes of wild strain tested. White = autosomes of *or Sc pr* standard testing strain.

bristles in the flies heterozygous for Scute, then the flies manifesting Smoky should have fewer bristles than those free of this gene. If such modifiers are present in the third or fourth chromosome, similar differences should be observed between orange and non-orange, and between Curly and non-Curly flies. The presence of modifiers of the dominance of Scute is clearly shown when one examines the mean number of bristles present

in the different classes of offspring obtained in the crosses *or Sc pr* ♀ ×*Sm or Cy*/Merritt ♂, *Sm or Cy*/Henshaw ♂, *Sm or Cy*/Grand Canyon ♂, and *Sm or Cy*/Big Horn ♂ (table 8). For example, in the offspring of the cross of *or Sc pr* ♀ ×*Sm or Cy*/Merritt ♂, the classes can be divided into two main groups, those having high mean bristle numbers, and those having low ones. Upon closer examination, one finds that all classes having low means contain the fourth chromosome marker *Cy*, irrespective of what other markers may or may not be present. On the other hand, all classes not marked by *Cy*, that is, containing the Merritt fourth chromosome, have high means. In other words, the gene, or genes, present in the Merritt strain which tend to suppress the dominance of Scute, and thus to produce many bristles in the Merritt/Scute hybrids, lie in the fourth chromosome of the Merritt strain.

TABLE 7

Mean number of bristles in homozygous Scute flies of the standard or Sc pr strain.

FEMALES				MALES			
M	±	m	n	M	±	m	n
1.357	±	0.09	168	1.98	±	0.16	68

In the cross of *or Sc pr* ♀ ×*Sm or Cy*/Henshaw ♂ the differences in the means of the various classes are hardly significant. One can not show any definite trend for any of the chromosomes. This may be due to the fact that heterozygous Scute in *or Sc pr*/Henshaw hybrids is completely dominant in the sense that heterozygous Scute and homozygous Scute would be alike in this background (compare tables 1 and 2 with table 7). Moreover, the modifiers in the *Sm*, *or*, and *Cy* chromosomes of the standard strain do not differ significantly from those in their respective homologues in the Henshaw strain. The slight differences in means of the various classes may be due to the presence of weakly opposing modifiers in the *Sm or Cy* chromosomes, or to the interactions of various combinations of these chromosomes with those of Henshaw.

An analysis of the Grand Canyon strain (table 8) shows that each of the autosomes of this strain contains some modifiers tending to suppress the dominance of Scute. The sum total of these modifiers suppresses Scute to such an extent that it has a mean number of bristles equal to that of Merritt. On the other hand, Big Horn (table 8) which gives a low mean, and does not have any important modifiers which differ from those of the *Sm or Cy* chromosome in suppressing the manifestation of Scute, may even have some actually enhancing Scute. These are located in the fourth

TABLE 8

Mean number of bristles present in offspring of or Sc pr ♀ × Sm or Cy/Merritt, Sm or Cy/Henshaw, Sm or Cy/Grand Canyon, Sm or Cy/Big Horn males.

	FEMALES		MALES	
	M±m	n	M±m	n
Cross to Merritt				
wild type	3.12±0.13	249	3.63±0.16	139
Sm	3.14±0.18	94	3.99±0.22	68
or	2.82±0.13	199	3.15±0.19	119
Cy	1.23±0.13	196	2.34±0.22	76
Sm or	2.97±0.22	66	3.46±0.25	44
or Cy	1.21±0.13	165	1.90±0.22	86
Sm Cy	1.60±0.15	131	1.87±0.17	106
Sm or Cy	1.58±0.23	72	1.65±0.19	63
Cross to Henshaw				
wild type	1.21±0.12	188	1.57±0.17	108
Sm	1.10±0.13	156	1.29±0.15	78
or	0.95±0.10	206	1.21±0.15	116
Cy	1.16±0.11	169	1.55±0.21	74
Sm or	1.33±0.19	91	1.28±0.20	32
or Cy	1.22±0.13	150	1.58±0.18	79
Sm Cy	1.29±0.11	187	1.71±0.18	91
Sm or Cy	1.61±0.16	114	1.58±0.20	62
Cross to Grand Canyon				
wild type	2.82±0.16	197	2.91±0.20	126
Sm	2.06±0.14	205	1.82±0.22	91
or	1.94±0.16	161	2.36±0.22	91
Cy	2.10±0.15	165	1.35±0.20	72
Sm or	1.76±0.15	132	1.75±0.28	49
or Cy	1.69±0.14	146	1.64±0.20	97
Sm Cy	1.78±0.15	208	1.74±0.16	106
Sm or Cy	1.57±0.16	121	1.48±0.20	67
Cross to big Horn				
wild type	1.43±0.12	166	1.80±0.14	157
Sm	1.58±0.15	160	1.85±0.26	61
or	1.57±0.13	186	1.40±0.14	137
Cy	2.01±0.16	169	1.99±0.18	102
Sm or	1.04±0.14	92	1.76±0.35	33
or Cy	1.76±0.15	173	1.62±0.19	107
Sm Cy	1.87±0.17	160	1.90±0.24	62
Sm or Cy	1.86±0.21	106	2.15±0.30	53

chromosome. This is shown by the fact that all classes having the marker *Cy* from the *Sm or Cy* strain have a higher mean number of bristles than do those having the Big Horn fourth chromosome. Thus the fourth chromosome of the Big Horn strain differs from that of the *Sm or Cy* strain in modifiers increasing the dominant effect of Scute.

TABLE 9

The χ^2 values for one degree of freedom for the comparisons of bristle numbers in the classes of flies shown in table 8 (further explanation in text).

CROSS	SEX OF OFFSPRING	COMPARISON		
		SMOKY-NON-SMOKY	ORANGE-NON-ORANGE	CURLY-NON-CURLY
or Sc pr ♀ ×	Females	0.3336	3.3244	498.6649
Sm or Cy/Merritt ♂	Males	6.3511	15.3373	221.6777
or Sc pr ♀ ×	Females	11.1117	0.1853	10.2736
Sm or Cy/Henshaw ♂	Males	0.0003	0.6229	5.7379
or Sc pr ♀ ×	Females	28.7180	41.0079	33.1835
Sm or Cy/Grand Canyon ♂	Males	25.6705	6.0986	69.5099
or Sc pr ♀ ×	Females	0.8731	3.5270	40.8005
Sm or Cy/Big Horn ♂	Males	6.1116	7.7835	5.8999

TABLE 10

The deviation χ^2 values for one degree of freedom for comparisons of various combinations of the bristle numbers in the classes of flies shown in table 8.

COMBINATION OF BRISTLE NUMBERS	SEX OF OFFSPRING	COMPARISON		
		SMOKY-NON-SMOKY	ORANGE-NON-ORANGE	CURLY-NON-CURLY
Merritt and Henshaw	Female	0.0425	2.1838	230.7472
	Male	4.9129	12.5665	100.2239
Grand Canyon and Big Horn	Female	16.8496	39.5555	0.0399
	Male	2.5467	14.5746	17.5445
Merritt and Big Horn	Female	2.5245	8.7078	150.2030
	Male	1.5832	24.6504	84.0996
Grand Canyon and Henshaw	Female	0.9086	26.3722	7.2538
	Male	12.8913	6.0955	21.0547
Merritt and Grand Canyon	Female	18.8365	33.9440	386.1411
	Male	34.4637	19.0469	278.1108
Henshaw and Big Horn	Female	2.1068	0.9153	48.6509
	Male	1.8823	6.6120	11.1457

Unfortunately, the data given in table 8 enable us to compare the various wild strains only to the standard one (*Sm or Cy*). In order to detect the similarities and differences between the effects of homologous chromosomes of the wild strains themselves (Merritt vs. Henshaw, for example)

it is necessary to use a more comprehensive statistical technique. According to the advice of DR. K. MATHER, the testing of the significance of the differences observed was made with the aid of two-by-two contingency tables, and the χ^2 values were obtained according to the formula: $\chi^2 = (a_1a_4 - a_2a_3)^2n/(a_1+a_2)(a_3+a_4)(a_2+a_3)(a_2+a_4)$ (FISHER 1928c). In this formula, a_1 is the total number of bristles present, and a_2 is the total number of bristles absent in a given class of flies (for example, in non-Curly females of the Merritt cross); a_3 and a_4 are the total numbers of bristles present and absent respectively in the flies of the class to be compared with the first (for example, in the Curly females from the same cross): n is equal to the sum of a_1, a_2, a_3, a_4. The resulting value of the χ^2 is a measure of the significance of the differences observed (FISHER 1928c). The χ^2 values for various comparisons made are presented in table 9. These values are for one degree of freedom. Looking up the probable significance of these values in FISHER'S table, the χ^2 of 2.706 has a probability of being a chance occurrence of one in ten, and a χ^2 of 6.635 of only once in one hundred trials. An examination of the females from the *Sm or Cy*/Merritt cross shows that the χ^2 value of the Curly-non-Curly comparison is the only one which is significant (table 9). Thus Merritt females differ from those of the standard testing strain in the fourth chromosome only. This is but a confirmation of the datum for Merritt given in table 8. In the female offspring of the *Sm or Cy*/Henshaw cross, the χ^2 values of *Sm* non-Smoky and Curly non-Curly are both significant. This indicates that there are modifiers in the second and fourth chromosomes of the Henshaw strain which suppress the dominance of Scute. The third chromosome is neutral. In the *Sm or Cy*/Grand Canyon cross the χ^2 values for all three classes of comparisons are significant. Thus in each of the autosomes tested there are factors suppressing the effects of Scute. The only significant deviation from the standard strain in the *Sm or Cy*/Big Horn cross is in the fourth chromosome. The above analysis shows that in two cases out of four examined, the modifiers affecting the dominance of Scute are located entirely in the fourth chromosome, in one case they are in the second and fourth chromosomes, and in only one case do we find modifiers in the third chromosome as well.

The data for males agree in general with those for the females. The main difference between the *Sm or Cy*/Merritt males and the standard strains is, as in the females, due to the fourth chromosome. The second and third chromosomes also show significant effects. These are not great enough, however, to account for the large suppression of Scute in hybrids with Merritt. The results for the *Sm or Cy*/Grand Canyon males are entirely in accord with those for the females; that is, the χ^2 values for the second, third and fourth chromosomes are all significant. An examination

of *Sm or Cy*/Big Horn males gives results slightly different from those of the females. In the males each chromosome has a significant modifying effect, whereas in the females, the only deviation from the standard strain is in the fourth chromosome.

A final test was made to compare the differences between the respective autosomal homologues of the four tested strains themselves. In order to do this, the number of bristles present in the corresponding classes of any two strains (Merritt and Henshaw, for example) were added together, and these combined values were used as single ones. Two-by-two contingency tables were constructed from these values and χ^2 were obtained in the same manner as above. These are deviation χ^2's and are given in table 10. They are subtracted from the total χ^2 values obtained by adding together the separately calculated ones from the two strains being tested (Merritt and Henshaw for example). The resulting heterogeneity χ^2 is an indication of the direct difference between the autosomes of the strains tested.

Unfortunately, the method first devised by DR. MATHER and used in this paper is not absolutely accurate. Since this paper was written, a more accurate method has been devised by him. The method used (the subtraction method) is accurate only when none of the χ^2 values is significant. In such cases where the deviation χ^2 is significant, the heterogeneity χ^2 is underestimated. Furthermore, discrepancies in the value of p, a measure of the proportion of bristle places where there are actually bristles present, further exaggerates the error. The method used counts these values in the classes tested to be the same. Unfortunately, this is not always true. Therefore, in a few extreme cases the deviation χ^2 is much overestimated and appears to give negative values for the heterogeneity χ^2. In spite of this, the analysis is accurate enough to indicate whether there is or is not a significant difference between the two chromosomes compared.

The results of the heterogeneity χ^2 values are given in table 11. As has been shown already (table 9), Merritt deviates very significantly from the females of the standard strain in the fourth chromosome only. Henshaw females differ from this strain in the second as well as in the fourth chromosome. One might surmise that Merritt females would differ directly from Henshaw females in these two autosomes, the second and fourth, and in this example, this is the case (table 11).

Grand Canyon females differ very significantly from the standard strain in all three autosomes. Big Horn females deviate from it in the fourth chromosome only (table 8). One can not definitely state in this case just how dissimilar these two strains are. It is safe to assume that their second and third chromosomes are not alike, but the fourth chromosomes might be similar if both differ from the standard strain in the same direction. On

the other hand, they might deviate from each other more than either one does from the standard strain, due to the fact that they differ in opposite directions. The latter possibility proves to be the case. Thus, the fourth chromosomes of these two strains are even less alike when compared to each other than if they are compared to the homologues in the *Sm or Cy* strain. The second and third chromosomes do not deviate as much from each other (table 11) as either does from the standard strain. This is due to both of them differing from the standard in the same direction.

Merritt and Big Horn females differ from the standard strain in their fourth chromosomes. Although the signs of the differences are alike, their fourth chromosomes are still very significantly dissimilar from each other (table 11). Here we have a very special case. Due to the overestimation of the deviation χ^2, the heterogeneity χ^2 value for the second and third chromosomes seems to be negative. Although this is not really the case, it is safe to assume that there is no significant difference between these two chromosomes.

Grand Canyon females and Henshaw females are unlike in all three tested autosomes. It is interesting to note, however, that the second and fourth chromosomes of these two strains deviate from the standard strain in opposite directions (table 11). The third chromosomes of these two strains differ from the standard strain in the same direction but they are still significantly dissimilar to each other. Although Grand Canyon also differs significantly from Merritt in all three autosomes, both of them deviate from the standard strain in the same direction as is shown by results given in table 11.

Henshaw and Big Horn females differ in their second autosomes (table 11). This is in accord with expectations (see table 9). It is especially interesting to note that although each strain varies significantly from the standard strain in the fourth chromosome, this difference disappears on direct comparison. In short, the fourth chromosomes of the Henshaw strain are not significantly different from those of the Big Horn strain. The third chromosomes are also alike. This example is a good one to show how insecure it is to compare two strains to each other through the medium of a standard third strain. The data for the males (table 11) on the whole lead to the same conclusions as those for the females.

PATTERN EFFECTS

As shown above, different strains of *D. pseudoobscura* produce hybrids with Scute having different numbers of bristles (tables 1 and 2). The question arises whether the Scute pattern is, or is not, changed in these strains, that is, whether any pattern effects are produced. The decrease of the total number of bristles in hybrids with one strain as compared with another

may be due to every bristle being removed proportionately more frequently in the first than in the second (lack of pattern effects); or else, a certain bristle, or set of bristles, may be affected disproportionately in different genotypes. The strains Merritt and Henshaw were studied in detail to detect the possible pattern effects. The four dorsocentral and the four scutellar bristles were counted in the hybrids between these

TABLE 11

The heterogeneity χ^2 values for one degree of freedom obtained by subtracting the deviation χ^2 from the total χ^2 (further explanation in text).

COMBINATION	SEX OF OFFSPRING	COMPARISON		
		SMOKY-NON-SMOKY	ORANGE-NON-ORANGE	CURLY-NON-CURLY
Merritt and Henshaw	Female	11.3928	1.4259	278.1914
	Male	1.3585	3.3937	127.1917
Grand Canyon and Big Horn	Female	12.7415	4.9794	73.9441
	Male	29.2354	−0.6025	57.8653
Merritt and Big Horn	Female	−1.2178	−1.8564	389.2624
	Male	10.8795	−1.5396	143.4780
Grand Canyon and Henshaw	Female	38.9212	14.8210	36.2033
	Male	12.7795	0.6260	54.1931
Merritt and Grand Canyon	Female	10.2152	10.3883	145.7073
	Male	−2.4421	2.2890	13.0768
Henshaw and Big Horn	Female	9.8780	2.7970	2.4232
	Male	4.2296	1.6944	0.4921

strains and Scute, the presence or absence of the anterior and the posterior dorsocentral and scutellar bristles being recorded separately for each individual. The data obtained are summarized in table 12.

The data were organized in four-by-four contingency tables, and the probable significance of any differences calculated by means of the χ^2 method. The formula used was $\chi^2 = S(a^2/m) - n$, where a represents the observed values, m represents the expected values (on the assumption that all four classes are equal), and n is the total number of bristles present. The comparison between Merritt and Henshaw gave a $\chi^2 = 7.82$ for three degrees of freedom. Although the method used slightly underestimates the difference, thus giving a minimum value for the χ^2, the significance of this difference is doubtful, since the probability of such a deviation occurring by chance is once in twenty trials. Merritt compared with the homozygous *or Sc pr* standard strain gave a $\chi^2 = 6.97$ for three degrees of freedom. This

is not significant, as the probability of such a value occurring by chance is between one in ten and one in twenty trials. Henshaw when compared to the *or Sc pr* strain gave a significant χ^2 value of 12.46.

The difference between the actions of Scute in Merritt and Henshaw hybrids is therefore essentially quantitative, the presence of a pattern effect being doubtful. Heterozygous Scute in the Merritt hybrid takes off bristles in much the same manner as does Scute in homozygous condition. There is, however, a perceptible qualitative difference between the action of Scute in Henshaw/*Sc* hybrids and its action in homozygous Scute.

TABLE 12

Number of bristles present in F_1 females of wild-type ♀ × or Sc pr ♂ crosses.

CROSS	DORSOCENTRAL		SCUTELLAR		NO. OF FLIES
	ANTERIOR	POSTERIOR	ANTERIOR	POSTERIOR	
Merritt ♀ × *or Sc pr* ♂	77	128	80	120	139
Henshaw ♀ × *or Sc pr* ♂	39	71	28	45	136
or Sc pr (Homozygous)	33	49	34	65	168

ARE THE DOMINANCE-SUPPRESSING MODIFIERS OF SCUTE SPECIFIC?

The presence of modifiers in various strains of *D. pseudoobscura* tending to suppress the manifestation of the semi-dominant mutation Scute has already been shown (tables 1 and 2). The question has been raised as to whether these modifying genes are of a non-specific nature, tending to increase the number of bristles present in flies in general, or whether they are specific, acting only in the presence of Scute itself. In order to distinguish between these two possibilities the following experiment was undertaken: Virgin females from a strain of race B of *D. pseudoobscura* containing the sex-linked recessives scarlet and scutellar were crossed to males of Merritt-3, Grand Canyon-2, Henshaw-3, and Big Horn-6; the scutellar bristles present in the F_1 males were counted.

The race-B mutant scutellar is sex-linked and recessive, and removes bristles only from the scutellum and the head, whereas the race-A mutant is an autosomal dominant which also removes the large dorsocentral bristles from the thorax. It seems logical to conclude that these two genes act in different ways to produce their effects. If the action of the Scute modifiers is a specific one for this mutant, one should expect no relation between the seriation of the same group of strains in hybrids with Scute as compared with their seriation in hybrids with scutellar. On the other hand, if these modifiers are of a general nature, acting solely to produce extra bristles, then one would expect to obtain parallel seriations from such an experiment.

The results of the experiment (table 13) are in accord with the assumption that the modifiers are specific. Merritt-2 and Grand Canyon-3 which act alike, producing high mean bristle numbers in hybrids with Scute (tables 1 and 2), are very significantly different from each other in their action on scutellar. Merritt has a high number of bristles in scutellar hybrids with it, but Henshaw-3 and Big Horn-6 (which have low mean numbers of bristles in Scute hybrids), also have mean bristle numbers of the same magnitude as Merritt. Furthermore, Grand Canyon has the lowest mean bristle number of the group tested with scutellar, yet in Scute hybrids it usually has one of the highest bristle numbers. Thus the modifiers tending to suppress the dominance of Scute in heterozygous form appear to be specific.

TABLE 13

Mean number of bristles present in F_1 males from cross of scutellar scarlet ♀ × Merritt-2, Grand Canyon-3, Henshaw-3 and Big Horn-6 ♂s.

STRAIN	n	M±m
Grand Canyon-3	242	0.56±0.04
Merritt-2	89	1.18±0.08
Henshaw-3	121	1.06±0.08
Big Horn-6	97	0.95±0.08

DISCUSSION

The purpose of the present work has been to collect experimental evidence bearing on the problem, of the nature and origin of dominance. As stated in the introduction, two distinct theories have been advanced in this field by FISHER (1928a, b, 1931, 1934) and by WRIGHT (1929a, b, 1934a, b,) and HALDANE (1930, 1932, 1933) respectively. FISHER'S theory attributes the development of dominance to a selection of specific modifying genes which tend to make each mutant heterozygote virtually indistinguishable from the wild-type. The theories of WRIGHT-HALDANE postulate rather a multiplicity of wild-type alleles which in connection with the rest of the genetic system, tend to make most of the frequently recurring mutations recessive.

The facts at hand show that the manifestation of Scute in hybrids with different wild strains of *D. pseudoobscura* is variable. Some strains such as Henshaw-3 and Big Horn-6, produce heterozygotes in which Scute is almost completely dominant; others, for example Merritt-2 and Grand Canyon-3, make the Scute heterozygotes decidedly intermediate between homozygous Scute and homozygous wild-type. Although no strain was found making Scute completely recessive, it is quite obvious that the species *D. pseudoobscura* is not uniform in its reaction to Scute. The variability observed shows no clear geographical trend; it looks as though

differences in the reaction to Scute may be found between strains from any region. If the evolutionary process be supposed to lead towards making the Scute mutation eventually recessive, this process is by no means completed.

The genetic causation of the variability in the degree of the dominance of Scute has been studied in four strains of race A and in one strain of race B of *D. pseudoobscura*. In every instance this variability proved to be due to modifying genes located in various chromosomes, more or less at random. There seems to be no tendency for such modifiers to be concentrated in the third chromosome which carries the locus of Scute itself. Moreover, there is no evidence, in the few cases studied, of the existence of a variety of wild-type alleles of Scute. Finally, evidence was obtained showing that the action of these modifiers appears to be specific for the mutant Scute. Therefore, our data, as far as they go, are in accord with expectations based on FISHER's theory. On the other hand, they can not be construed as in any way contradictory to the alternative theory of WRIGHT-HALDANE. It is possible that the development of dominance is due to a selection of wild-type alleles of the mutant genes themselves as well as to that of modifiers at other loci. Moreover, presence of such modifiers is not in itself a proof that the dominance of the wild-type condition over the mutant will eventually be attained by their selection.

ACKNOWLEDGMENTS

I wish to express my appreciation for the suggestions and aid of PROFESSOR TH. DOBZHANSKY and PROFESSOR A. H. STURTEVANT who made this work possible, and to DR. K. MATHER for his help in the statistical treatment of the data and particularly of those in the Smoky-orange-Curly, and in the pattern-effect experiments.

SUMMARY

1. A study was made of various strains of race A and B of *D. pseudoobscura*, and their reactions to the semi-dominant mutation Scute. It was found that some strains gave high bristle numbers in hybrids with Scute (Merritt and Grand Canyon), others gave low bristle numbers (Henshaw and Big Horn), and still others were intermediate (tables 1 and 2).

2. No relation was found between the degree of dominance of Scute in hybrids with a given strain and the geographical origin of the latter.

3. In tables 1 and 2, the females of the various strains are arranged in descending order with respect to the number of bristles present. In general, the males agree with this seriation. On occasion, they do not. Those cases where the male disagrees with the female seriation suggest the presence of modifiers in the Y chromosomes of the wild strains.

4. The existence of modifiers located in the X chromosome is suggested in those crosses of wild-type ♀ × *or Sc pr* ♂ where the male offspring disagree with the normal female seriation.

5. Several strains were analyzed to see if wild-type alleles of Scute of different strengths were the reason, or at least a part of the reason, for the different behavior of Scute in hybrids with these strains. No difference was found between the wild-type alleles of Scute present in race A and race B strains, which had been made isogenic except for a region of the chromosome around the locus of Scute. In another test, the widely unlike strains of race A, Merritt and Henshaw, proved to show no difference as far as their Scute loci were concerned.

6. A more complete analysis of two of the strains with high bristle numbers (Merritt and Grand Canyon), and two strains with low bristle numbers (Henshaw and Big Horn), shows that the main differences in their reactions to Scute are due to the presence of modifying genes in the autosomes (table 11). In one case (Merritt) the modifiers are mainly in the fourth chromosome, in another (Grand Canyon) they are found scattered in all of the autosomes tested. In Henshaw there are no very strong modifiers, although some weak ones exist in the second and fourth chromosomes. Big Horn has modifiers in the fourth chromosome, and some rather weak ones in the third.

7. A study was made to see if the pattern in which Scute removes the bristles is affected in hybrids with Merritt and Henshaw. It was found that the Scute pattern was not significantly altered in hybrids with Merritt, but it was in hybrids with Henshaw.

8. A final test was made to determine the degree of specificity of these Scute modifiers. It was found that the effects produced seemed to be specific for this mutant, and were not general modifiers tending to increase the number of bristles per se.

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GENETIC STUDIES WITH FACTORS IN THE TENTH CHROMOSOME IN MAIZE[1]*

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Received December 1, 1938

INTRODUCTION

THE shortest chromosome of the haploid set of ten in maize has been designated as chromosome 10. In addition to its relatively short length it is characterized at pachytene by the position of the spindle attachment region which divides the chromosome into two unequal arms in the ratio of 2.6:1, and the presence of deeply staining chromomeres adjacent to the spindle attachment region (McCLINTOCK 1931, and figure 1C). Owing to its short length, chromosome 10 often is found lying free from the other chromosomes at pachytene and therefore lends itself well to cytological examination. The association of a group of linked genes with chromosome 10 was first accomplished by McCLINTOCK and HILL (1931) who found trisomic ratios for the *R* locus when chromosome 10 was in triplicate. This association between linkage group and chromosome has been subsequently confirmed through cytogenetic studies of deficiencies involving genetic factors in the tenth linkage group (McCLINTOCK 1931, STADLER 1933, V. H. RHOADES 1935) and also through cytogenetic studies of reciprocal translocations involving chromosome 10 (ANDERSON 1935).

The summary of linkage data in maize by EMERSON et al. (1935) lists twenty-two genes in the tenth linkage group. However, the linear sequence of only three of these genes could be determined because of the paucity of three-point linkage data. The linear sequence, with the corresponding map distances, of the nl_1 (narrow leaf), g_1 (golden plant color), and *R* (aleurone and plant color) loci was given as nl_1 18 g_1 14 *R*. The data reported in this paper place two previously unreported genes, l_8 (luteus seedling) and sp_2 (small pollen), in the tenth linkage group and fix the map position of *Rp* (resistance to *Puccinia sorghi* Schw.), *li* (lineate striped leaves), and d_7 (dwarf plants). The map length is extended from 32 to 84 units.

LINKAGE STUDIES WITH THE *Rp* GENE

MAINS (1931) demonstrated that resistance to physiologic form 3 of

* A part of the cost of the accompanying figures is met by the Galton and Mendel Memorial Fund.

[1] Contribution from the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

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Puccinia sorghi Schw. is determined by a single dominant gene in maize. This gene, designated *Rp*, was associated with the tenth linkage group by the trisomic method, and placed in the distal one-fourth of the short arm of chromosome 10 by cytological examination of plants carrying X-ray induced deficiencies for *Rp* (V. H. RHOADES 1935).

To fix the position of *Rp* in the linkage group, studies have been made using cultures carrying *Rp* and other genes of the tenth linkage group. Individuals heterozygous for *Rp li* g_1 were backcrossed by a triple recessive stock. The seedlings in the backcross population were inoculated with spores of physiologic form 3 of *Puccinia sorghi* Schw. The seedling population consisted of 374 resistant, *Rp* +, individuals and 379 susceptible, + +, individuals. The *li* character cannot be recognized in the seedling stage so the 374 resistant seedlings were transplanted to the field. The susceptible plants were not saved because of the danger of an epidemic of rust in other genetic material. The data obtained from the 366 survivors of the 374 resistant seedlings are presented in table 1. The linear order of the three loci determined from the data in table 1 is *Rp li* g_1.

TABLE 1

Linkage data from the cross of $\frac{Rp\ +\ +}{+\ li\ g_1} \times +\ li\ g_1$. *Data were obtained only from the resistant (Rp+) plants.*

F_1 GENOTYPE	PARENTAL COMBINATION	REGION 1	REGION 2	REGIONS 1 & 2	TOTAL
$\frac{Rp + +}{+\ li\ g_1}$	223	90 *24.5%*	40 *10.9%*	13 *3.6%*	366

Backcross data were also obtained for the *Rp* g_1 *R* loci. Fortunately, the g_1 and *R* characters could be recognized in seedlings and aleurone, respectively, so resistant and susceptible individuals were classified for both g_1 and *R*. These data are presented in table 2.

The linear order from the data in table 2 is undoubtedly *Rp* g_1 *R*. The data of tables 1 and 2 give the sequence and intervening map distance of the four loci as *Rp* 28 *li* 15 g_1 14 *R*.

MCCLINTOCK (1931) found the entire long arm of chromosome 10 missing in a deficiency which included the *R* locus. STADLER (1933) studied a deficiency which included the distal 22 percent of the long arm and which included the locus of *R* but not that of g_1. The observations of V. H. RHOADES (1935) place *Rp* near the distal end of the short arm. The physical locations of *Rp* and *R* in chromosome 10 are shown graphically in figure 2. Some error may exist because of the unproved assumption that these deficiencies are strictly terminal.

SINGH in EMERSON et al. (1935) reported 27 percent recombination between d_7 (dwarf plant) and R. He also reported 35 percent recombination between d_7 and g_1 in a small population consisting of 109 plants. These data of SINGH's indicate that the order is g_1 R d_7. If this order (based on

TABLE 2

Linkage data from the cross of $\frac{Rp\ +\ +}{+\ g_1\ R} \times +\ g_1\ +$. *Data were obtained from both resistant and susceptible plants.*

F_1 GENOTYPE	PARENTAL COMBINATIONS	REGION 1	REGION 2	REGIONS 1 & 2	TOTAL
$\frac{Rp + +}{+ g_1 R}$	179 161	109 114 *34.2%*	31 30 *9.3%*	16 13 *4.4%*	653

the meager g_1 d_7 data) is misleading and d_7 lies to the left of g_1 it should show close linkage with Rp because the recombination percentage for Rp g_1 was 35.4. F_2 data on this possible linkage were therefore obtained and these are presented in table 3. There is no indication of linkage between

TABLE 3

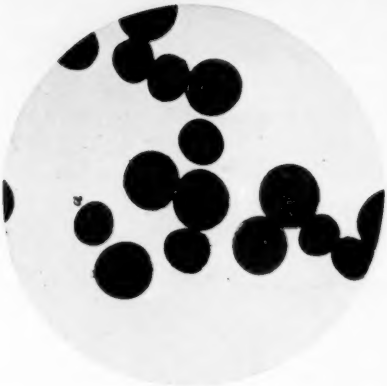
Linkage data from the selfing of $\frac{Rp\ +}{+\ d_7}$ *plants.*

LINKAGE PHASE	NO. OF INDIVIDUALS					RECOMBINATION PERCENT
	XY	Xy	xY	xy	TOTAL	
Coupling	409	127	133	41	710	50

Rp and d_7, so d_7 must fall to the right of R as SINGH's data suggested. If we accept SINGH's data on the R d_7 linkage as reasonably accurate, the sequence with the intervening map distances of the Rp li g_1 R d_7 genes is as follows: Rp 28 li 15 g_1 14 R 27 d_7. The length of this genetic map is 84 units.

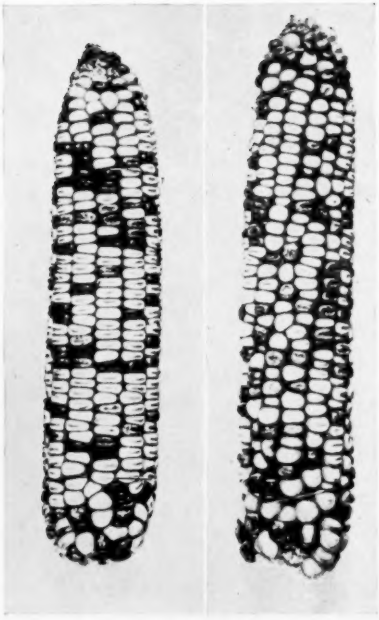
LINKAGE STUDIES WITH THE l_8 AND sp_2 GENES

In 1933 seed from selfed plants of open-pollinated varieties of maize was obtained from DR. R. G. WIGGANS of Cornell University. The progeny obtained from one of these selfed ears consisted of 42 green and 49 luteus or yellow seedlings. The luteus seedlings being devoid of chlorophyll died in the seedling stage. The high percentage of luteus plants suggested that some unusual genetic situation was involved so twenty-



1A

1C



1B

FIGURE 1

two of the green plants were self-pollinated. The ensuing progenies from each of the twenty-two selfed plants had in every case a high percentage of luteus seedlings. The combined progenies from the twenty-two ears had a total of 1,906 plants of which 1,251 or 65.6 percent were luteus. Subsequent studies showed that the green plants which threw the abnormally high luteus ratio were heterozygous for a single luteus gene and for a gene closely linked with it which adversely affects the survival of both male and female gametes. The luteus gene was designated luteus-8

TABLE 4

Linkage data from crosses of $+\ li\ g_1\ +\ \times\ \frac{+\ li\ \ g_1\ \ R}{sp_2\ +\ +\ +}$ *where heterozygous plants were used as the male parents. The classification for* sp_2 *was made by pollen examination. If a plant was* $++$ *all grains were of normal size whereas half were small when the plant was* $+sp_2$.

CONSTITUTION OF CHROMOSOMES FROM MALE PARENT																	
o	o	1	1	2	2	3	3	1–2	1–2	2–3	2–3	1–3	1–3	1–2–3	1–2–3		
+	sp_2	+	sp_2	+	sp_2	+	sp_2	+	sp_2	+	sp_2	+	sp_2	+	sp_2		
li	+	+	*li*	*li*	+	*li*	+	+	*li*	*li*	+	+	*li*	+	*li*		
g_1	+	+	g_1	+	g_1	g_1	+	g_1	+	+	g_1	+	g_1	g_1	+		
R	+	+	*R*	+	*R*	+	*R*	*R*	+	*R*	+	*R*	+	+	*R*		
1209	30	6	3	73	1	149	14	0	0	7	0	0	1	1	0		
		0.6%		5.0%		10.9%		0.0%		0.5%		0.07%		0.07%			
		(1)		(2)		(3)		(1–2)		(2–3)		(1–3)		(1–2–3)			

$\Sigma = 1{,}494$

$sp_2\ li = 0.7\%$ $\qquad$ $li\ g_1 = 5.5\%$ $\qquad$ $g_1\ R = 11.5\%$

Disregarding all multiple crossovers the recombination percentage between sp_2 and $R = 16.6$.
The order is $sp_2\ li\ g_1\ R$

(l_8) and the gamete gene was called sp_2 (small pollen-2) since it had a visible effect upon the size of the pollen grains (figure 1 A). The constitution of the green plants was $sp_2+/+l_8$. There is very little crossing over between these two loci. Pollen grains with the sp_2 gene rarely function in competition with grains carrying the normal allele, and plants homozygous for the l_8 factor are lethal. There is, practically, only one type of functional pollen; and of the two zygotic classes produced one is homozygous for l_8 and the other is heterozygous, as was the parent plant, for the sp_2

FIGURE 1—A. A group of pollen grains from a plant heterozygous for sp_2. The larger grains have the normal allele while the smaller have the sp_2 allele. B. The ear on the right was produced on a plant heterozygous for sp_2 and shows the partial sterility caused by the abortion of some of the sp_2 ovules. The sib ear to the left illustrates the full set of seed produced by plants homozygous for the normal allele. C. Photomicrograph at early pachytene of the two paired chromosomes 10 (marked by arrow) in a microsporocyte from a plant heterozygous for sp_2. No deficiency is visible here nor were any found in many other clear figures. The spindle attachment regions are visible as the translucent bulge lying above the arrow.

and l_8 genes. This class of plants would again produce more than 50 percent of luteus when selfed because sp_2 has a semilethal effect upon eggs carrying it and they do not all function. The above behavior where the heterozygotes form the only surviving class is essentially similar to the case of balanced lethals first described by MULLER (1918) for the Beaded wing situation in Drosophila.

Linkage studies placed the l_8 and sp_2 genes in chromosome 10. When $sp_2+/+\ l_8$ plants were pollinated by an $li\ g_1\ R$ stock two classes of F_1 plants resulted. Those F_1 individuals of $sp_2\ +\ +\ +/+\ li\ g_1\ R$ constitution were backcrossed reciprocally to $li\ g_1\ +$ lines and four-point linkage data obtained. These data are presented in tables 4 and 5.

TABLE 5

Linkage data from cross of $\frac{+\ li\ g_1\ R}{sp_2+\ +\ +} \times +\ li\ g_1\ +$ *where heterozygous plants were used as female parents. The classification for* sp_2 *was made by pollen examination. If a plant was* $+\ +$ *all pollen grains were normal size while half the grains were small when the plant was* $+\ sp_2$.

CONSTITUTION OF CHROMOSOMES FROM FEMALE PARENT															
0	0	1	1	2	2	3	3	1–2	1–2	2–3	2–3	1–3	1–3	1–2–3	1–2–3
+	sp_2	+	sp_2	+	sp_2	+	sp_2	+	sp_2	+	sp_2	+	sp_2	+	sp_2
li	+	+	*li*	*li*	+	*li*	+	+	*li*	*li*	+	+	*li*	+	*li*
g_1	+	+	g_1	+	g_1	g_1	+	g_1	+	+	g_1	+	g_1	g_1	+
R	+	+	*R*	+	*R*	+	*R*	*R*	+	*R*	+	*R*	+	+	*R*
432	243	4	5	30	19	59	27	0	0	5	2	0	1	0	0
		1.1%		5.9%		10.4%		0.0%		0.8%		0.1%		0.0%	
		(1)		(2)		(3)		(1–2)		(2–3)		(1–3)		(1–2–3)	

$\Sigma = 827$

$sp_2\ li = 1.2\%$ $\quad li\ g_1 = 6.8\%$ $\quad g_1\ R = 11.4\%$.

Recombination percentage $sp_2\ R = 17.4$

The order is $sp_2\ li\ g_1\ R$

The data in table 4 show that when a mixture of normal and sp_2 pollen was used only 3.3 percent of the grains effecting fertilization were of the sp_2 class although 50 percent was expected in the absence of competition. Forty-nine of the 1,494 plants tested carried the sp_2 gene. With no selection or inviability the number of sp_2 plants should have been equal to the normal or + individuals of which there were 1,445. In other words, 96.6 percent of the sp_2 pollen was unable to compete successfully against normal-sized pollen. The reason for this is unknown. The sp_2 grains although smaller are well filled with starch; they contain two normal sperm nuclei at the time of anthesis; and they germinate as readily on fresh silks as do normal-sized grains. It is possible that owing to their smaller volume they lack sufficient starch and food reserves to supply the energy neces-

sary for the pollen tube as it grows down the long silks. However it seems more likely in view of MANGELSDORF'S (1931) results, where he greatly increased the number of functioning *sp*$_2$ pollen grains by mechanically separating them from the larger grains, that the inability of the *sp*$_2$ grains to compete against normal pollen is caused by some handicap of a physiological nature such as a slower rate of pollen tube growth.

The linkage data in table 4 show that the order is *sp*$_2$ *li* *g*$_1$ *R*. An unusual feature of the data is the great reduction in the amount of crossing over in the *li* *g*$_1$ interval where only 5.5 percent of recombination was found as compared with 15 percent found in the controls. The crossover value for the *g*$_1$ *R* region is only slightly less than the control value of 14 percent and the difference may not be significant. A similar decrease was found in these two regions when the F_1 plants were used as female parents in a backcross test as is shown by the data in table 5.

The data in table 5 show that 35.8 percent of the functioning eggs are *sp*$_2$. With no infertility or inviability equal numbers of *sp*$_2$ and + eggs are expected. Actually only 297 of the 827 functioning eggs were *sp*$_2$. Therefore, 44 percent of the *sp*$_2$ eggs are inviable. Since in maize it is the basal member of the linear series of four megaspores which always develops into the embryo sac there can be no competition between the *sp*$_2$ and + eggs as is the case between the two classes of pollen. The deficiency of *sp*$_2$ eggs must be due to a semilethal effect of the *sp*$_2$ gene. Since only 56 percent of the *sp*$_2$ ovules develop into mature seeds, we should and do find on ears of + *sp*$_2$ constitution evidence of partial sterility caused by the failure of certain of the *sp*$_2$ ovules to mature (figure 1 B). No attempt has been made to determine if there is a one to one relationship between the sterility on ears heterozygous for *sp*$_2$ and the deficiency of *sp*$_2$ eggs but it is likely that such a relationship exists.

The data in tables 4 and 5 agree in showing that the order is *sp*$_2$ *li* *g*$_1$ *R*. The amount of recombination between *sp*$_2$ and *li* is one percent. There is no doubt, however, that the *sp*$_2$ gene reduces the frequency of crossing over in regions adjacent to its locus. It may be expected, therefore, that a locus to the left of lineate which would give as much as five or six percent of crossing over with the *li* locus in stocks with normal crossing over will actually lie between the *sp*$_2$ and *li* loci.

The *sp*$_2$ gene was first detected by its close linkage with *l*$_8$ and the consequent distorted ratios found for the luteus character. In order to arrive at some conception of the amount of crossing over between *sp*$_2$ and *l*$_8$, 238 green plants derived from selfing *sp*$_2$ +/+ *l*$_8$ individuals were self-pollinated and progenies grown from the resulting seed. Two hundred thirty two of the progenies had a high percentage of luteus. It follows that they were of *sp*$_2$ +/+ *l*$_8$ constitution and that both of the chromosomes were

non-crossovers. One progeny had only green seedlings. It was found to be of + +/sp_2 + constitution and the + + chromosome was evidently the result of a crossover between the two loci. In five of the selfed progenies there was a ratio of three green : one luteus. These ears must have come from plants of + +/+ l_8 heredity, and the + + chromosome was thus the consequence of a crossover between sp_2 and l_8. The 238 tested individuals had a total of 476 chromosomes. Six of these were crossover types; this result gives a crossover percentage of 1.3. If the sp_2 eggs functioned as frequently as the normal, we would expect two + +/+ l_8 individuals to one + +/sp_2+. However, because of the lower frequency with which sp_2 eggs survive, the theoretical ratio is greater than 2:1. The observed numbers were 5:1.

The distorted ratios found for those loci linked with sp_2 is well illustrated by the following data. When plants of sp_2 R/+ + constitution were used as the pollen parent in backcrosses with R-testers there were 516 R to 2,163+kernels although equality was expected. The R class comprises 19.3 percent of the total. In the reciprocal cross there were 388 R to 525+seeds. The higher percentage of R seeds is due, of course, to the fact that a considerable percentage of sp_2 eggs are fertile and give rise to mature kernels. When the same series of crosses was made with the two loci in coupling, there resulted 832 R to 643+kernels when sp_2 +/+ R individuals were used as the female parent in backcrosses. The reciprocal cross produced 3,655 R to 834 + or a percentage of 18.6 + kernels. Since we know from the data in table 4 that there is 16.6 percent of recombination between sp_2 and R and that 3.3 percent of the functional pollen is sp_2, the expected percentage of R or +, depending upon the linkage phase, is 18.7. The observed values of 19.3 and 18.6 percent are in very good agreement.

TABLE 6

Linkage data from the selfing of $\frac{+\ R}{l_8\ +}\ \frac{C}{+}$ *plants. There was a ratio of 9 colored: 7 colorless aleurone seeds as both C and R were segregating.*

LINKAGE PHASE	NO. OF INDIVIDUALS XY	Xy	xY	xy	TOTAL	RECOMBINATION PERCENT
Coupling	4,663	2,449	631	1,879	9,622	19

The location of the sp_2 locus could be ascertained with considerable accuracy because it was possible to obtain backcross data. Unfortunately it was impossible to obtain backcross data for linkage tests involving the l_8 factor as it is lethal when homozygous. Data have been presented which show a close linkage between sp_2 and l_8. It would be expected therefore

that those loci in chromosome 10 which showed linkage with sp_2 would also show approximately the same linkage with l_8. Because of the fact that the *R* gene affects aleurone color and the *R* and + classes can be separated before planting the seed, it was possible to obtain satisfactory data on the *R* l_8 linkage. These data are presented in table 6. They show 19 percent of recombination between *R* and l_8. This percentage of recombination indicates that l_8 would lie between *li* and g_1, since the g_1 *R* interval is 14 units and the *li* g_1 interval is 15 units. That this probably is the correct location for the l_8 locus is shown by the data in table 7 obtained by the selfing

TABLE 7

Linkage data from the selfing of $\frac{+\ l_8\ +\ +}{li\ +\ g_1\ R}\ \frac{C}{+}$. *There was a ratio of 9 colored : 7 colorless aleurone seeds since both C and R are segregating. The classification for luteus was made in the seedling stage and the excess of plants under the* L_8 *column over the sum of the* G_1 *and* g_1 *classes is due to the failure of all the non-luteus seedlings to survive to maturity.*

COLORED ALEURONE						COLORLESS ALEURONE					
+	+	*li*	*li*	L_8	l_8	+	+	*li*	*li*	L_8	l_8
+	g_1	+	g_1	—	—	+	g_1	+	g_1	—	—
851	95	60	446	1,561	181	548	34	26	174	825	672
	G_1	g_1		*Li*	*li*			l_8 *R*=17% recombination			
	1,485	749		1,528	706						

of + l_8 + +/*li* + g_1 *R* plants. These data show that there is 17 percent of recombination between *R* and l_8. The location of l_8 with respect to *li* and g_1 can only be ascertained by the relative effect of l_8 on the ratios of *li* and g_1. If, for example, l_8 is completely linked with g_1, a ratio of 2+:1 g_1 is is expected in F_2; whereas a 3:1 ratio is expected with independence. As the intensity of linkage between l_8 and g_1 decreases, the ratio of greens to goldens will range from 2:1 to 3:1. The data show a greater distortion of the g_1 ratio than of the *li* ratio and accordingly suggest that l_8 lies closer to g_1 than to *li*. It should be recognized that linkage data of this type are very unsatisfactory and little reliance can be placed on the order or location of loci determined solely from this type of linkage experiment. The greater distortion of the g_1 ratio is, however, in accord with expectancy, since we know that l_8 must lie to the left of *R* from the sp_2 l_8 linkage, and the 17 percent of recombination between l_8 and *R* would place l_8 considerably closer to g_1 than to *li*. The very close linkage between sp_2 and l_8 is most likely due to the decrease of crossing over found when sp_2 is heterozygous. It can be argued with some degree of assurance that the order is sp_2 *li* l_8 g_1. While the data in table 7 do not lend themselves to an exact determination of the amount of crossing over between *li* and g_1, there is

no indication here of the reduction in the *li* g_1 region found when the sp_2 allele was involved.

The available data place l_8 approximately 5 units to the left of g_1. SINGH reported 35 percent of recombination between g_1 and d_7. Since d_7 must lie to the right of *R* the expected amount of recombination between l_8 and d_7 should be in the neighborhood of 40 percent. The data in table 8 exhibit a recombination percentage of 47 between these two loci.

TABLE 8

F_2 linkage data derived from the selfing of $\frac{+\ d_7}{l_8\ +}$ *individuals.*

GENES	LINKAGE PHASE	NUMBER OF INDIVIDUALS XY	Xy	xY	xy	TOTAL	PERCENTAGE OF RECOMBINATION
$l_8\ d_7$	R S	1,411	425	488	117	2,441	47

With the exception of the doubtful linkage between l_8 and d_7 no data have been presented in this paper which show that d_7 belongs in the tenth linkage group. There can be no doubt, however, of the statistical significance of SINGH'S data for the *R* d_7 linkage and since our d_7 stocks came from SINGH there is no reason to believe that some other dwarf gene was segregating in our linkage tests. If we accept, provisionally at least, the inclusion of d_7 in the tenth chromosome, the linear order and map positions for the genes reported in this paper are as shown in figure 2.

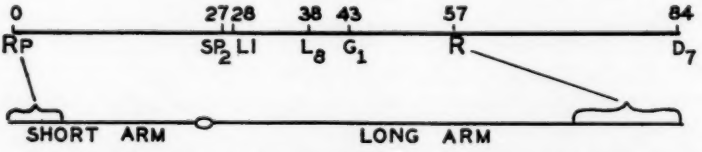


FIGURE 2.—The genetic map of chromosome 10 is shown in the upper part of the figure. The lower part illustrates the locations of the *Rp* and *R* genes in chromosome 10 determined from studies of deficiencies involving these loci. The spindle attachment region is represented by the ellipse.

DISCUSSION

Throughout the presentation of the linkage data on the location of sp_2, it has been assumed that the genetic condition responsible for the several effects was a point mutation at the sp_2 locus. This is a justificable assumption for the purposes of linkage. However, in 1933 STADLER published an illuminating paper on a haplo-viable deficiency in maize which has an effect strikingly similar to that of sp_2 factor reported here. A deficiency for the distal 22 percent of the long arm of chromosome 10 was produced through irradiation with X-rays. Pollen with this deficient chromosome

was smaller than the normal grains and incapable of germination, although some starch was present. There was also a high mortality among eggs carrying this deficiency, but in some ears approximately 30 percent of the deficient megaspores developed sufficiently well to permit seed production when fertilized by normal pollen. The effect of the sp_2 gene is not as severe for we have seen that three percent of the sp^2 pollen functions in competition with normal grains and that 56 percent of the sp_2 eggs develop into mature seed. Data have been presented which show that in addition to affecting the viability of the gametophytes, the sp_2 factor markedly reduces crossing over when it is heterozygous. Since, in STADLER's deficiency, the loss of nearly one-fourth of the long arm of chromosome 10 produced effects so similar to those found for sp_2, it seemed possible that a cytological examination of the sp_2 chromosome might show some chromosomal abnormality, as a deficiency. Cytological studies at pachytene were made of microsporocytes from numerous plants heterozygous for sp_2, but no evidence was found of structural dissimilarity between the two chromosomes 10. Figure 1 C is a microphotograph of the two paired chromosomes 10 from a plant heterozygous for sp_2 and no indication of any irregularity can be seen. It is possible that sp_2 is a deficiency so minute that our present cytological technique is not sensitive enough to disclose its presence. If the sp_2 condition is actually the result of an undetectable deficiency, and for the present we have no way of deciding between such a deficiency and a so-called point mutation, it is of interest to note that STADLER predicted such an event, for he says in discussing his haplo-viable deficiency "In this instance it may be demonstrated cytologically that the 'mutant allelomorph' of *R* is not a gene but the absence of a rather long chromosome segment. Its cytological identification however is made possible by the unusual advantages of the pachytene technique; in species in which this is not applicable a deficiency of equal length probably could not be cytologically identified. The interpretation of the induced variation as mutation or deficiency would then be wholly arbitrary. Ordinarily such a variation is regarded as a gene mutation unless there is genetic or cytological evidence indicating deficiency. Moreover, even in the pachytene chromosomes, deficiencies of much shorter length or less favorable location probably would be undetectable. Stretching and illegitimate pairing tend to mask slight inequalities of the synapsed chromosomes, particularly in non-terminal deficiencies. It is probable therefore that the shortest induced deficiencies may present a normal appearance in the pachytene chromosome. These also are the cases which would be least likely to be identified as deficiencies by genetic evidence."

STADLER found that his deficiency included the locus of *R* but not of g_1. Since sp_2 is to the left of g_1 it likewise should not be included, but no tests

on this point have been made. Theoretically it should be possible to obtain an individual carrying both STADLER'S deficiency and sp_2. The pollen from such a plant should all be smaller than normal but should consist of two distinct types since the effect of sp_2 is not as pronounced as STADLER'S deficiency on pollen size and on the amount of starch deposited. If any normal-sized pollen were found it would be due to crossing over between sp_2 and the right end of the deficient chromosome.

Data on another situation have been reported in maize (MANGELSDORF 1931, 1932, SINGLETON 1932) which are strikingly similar to the sp_2 data. These investigators found that distorted ratios for the + *su* pair, located in chromosome 4, were produced by linkage of the *su* locus with a gene, or genetic condition, producing tiny pollen. This gene has been called sp_1; and MANGELSDORF (1932) reports that only slightly more than one percent of the functional pollen from + sp_1 plants is sp_1. He showed, however, that sp_1 pollen was able to function when not forced to compete with normal pollen. It is possible to calculate from MANGELSDORF'S data published in EMERSON et al. (1935) that 38.8 percent of the sp_1 eggs are infertile. It will be recalled that approximately three percent of the functioning pollen from + sp_2 plants was sp_2 and that 44 percent of the sp_2 eggs did not develop into mature kernels. These values are surprisingly similar to those obtained for sp_1. The slightly higher survival values for both types of gametophytes indicate that the effect of sp_2 is not quite as severe as is sp_1. SINGLETON (1932) states that cytological examination of the chromosomes in + sp_1 plants at the reduction division disclosed no chromosomal irregularity. It would appear from MANGELSDORF'S data in EMERSON et al. that there was no reduction in crossing over when sp_1 was heterozygous, since he lists recombination values of 36 and 33 percent for the *su Tu* interval while the standard value for this region is 29 percent. In this respect then sp_2 may differ from sp_1.

MCCLINTOCK (1938) showed that eggs carrying a deficiency which included one-twentieth of the length of chromosome 5 were able to function in 7.2 percent of the cases. Pollen of the same constitution was smaller than normal, partially filled with starch, and incapable of accomplishing fertilization in the absence of competition with normal pollen. MCCLINTOCK also studied a second deficiency which involved one-seventh of the length of chromosome 5. This deficiency was lethal in both eggs and pollen.

BURNHAM (1932) found in his studies with a reciprocal translocation in maize involving chromosome 1 and chromosome 6 that eggs having a duplication for a considerable portion of chromosome 1 and deficient for the terminal chromomere of the satellite of chromosome 6 were able to develop when fertilized by sperm with a full complement of genes. Pollen

of the same constitution was non-functional although well filled with starch. CLARKE and ANDERSON in 1935 reported a similar situation with a translocation which also involved the satellite region of chromosome 6.

The failure to find sp_2 associated with a visible deficiency is not surprising in view of some recent work in Drosophila by B. M. SLIZYNSKI (1938), HELEN SLIZYNSKA (1938), and HOOVER (1938). These investigators made careful studies of salivary chromosomes carrying genetic lethal changes or mutations. In less than half of the cases (B. M. SLIZYNSKI) could a minute deficiency be associated with the lethal condition known to exist by genetic tests. SLIZYNSKI states that the failure in some cases to find deficiencies does not constitute definite proof that deficiencies do not exist in that particular material. It is merely negative evidence. However, if we are not able to discriminate cytologically between minute deficiencies and intragenic changes, the classification of a lethal mutation is arbitrary even though we may favor the deficiency as the causal agent because many lethal changes are known to be associated with deficiencies. It should be remembered, however, that DEMEREC and HOOVER (1936) found a visible cytological deficiency which had no lethal effect. It is quite probable, as HOOVER states that " . . . genetic-physiological lethals and cytological deficiencies are not necessarily synonymous."

SUMMARY

Linkage data are presented which place the gene for resistance to rust (*Puccinia sorghi* Schw.) in the genetic map of chromosome 10. These data also determine the linear order and map position of the *li* (lineate) and d_7 (dwarf) loci.

Two previously undescribed characters are reported. One of these, designated sp_2 (small pollen), has a semilethal effect upon the male and female gametophytes. Pollen grains carrying the sp_2 factor do not differ visibly from normal grains except that they are smaller; yet 96.6 percent of sp_2 grains are unable to compete successfully with normal pollen. Forty-four percent of sp_2 eggs are infertile and do not develop into mature kernels. Crossing over is reduced in adjacent regions when sp_2 is heterozygous. Linkage data place sp_2 approximately one crossover unit to the left of *li*. Cytological studies at pachytene disclosed no chromosomal irregularity in + sp_2 plants. Linkage data are presented which locate the other new gene l_8 (luteus) between *li* and g_1.

The linkage data extend the genetic map for chromosome 10 from 32 to 84 units. The sequence and map positions as determined from these data are

Rp	sp_2	*li*	l_8	g_1	*R*	d_7
0	27	28	38	43	57	84

Earlier cytological observations had placed the *Rp* gene in the distal one-fourth of the short arm of chromosome 10 (V. H. RHOADES), and the *R* locus in the distal twenty-two percent of the long arm (STADLER).

ADDENDUM

Extremely pertinent evidence on the genetic nature of lethal mutations has recently been presented by SUCHE, PARKER, BISHOP and GRIFFEN (Genetics **24**: 88, 1939). These investigators found that several lethal point mutations and several lethals associated with chromatin rearrangements could be reversed to a non-lethal condition by irradiation. The fact that some lethals revert to a non-lethal condition is strong evidence that these lethals are not due to deficiencies. There is no doubt but that more than one mechanism is responsible for all types of lethal mutations, that is, some are deficiencies, some point mutations or position effects.

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